

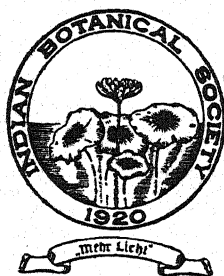
THE JOURNAL

OF THE

Indian Botanical Society

EDITED BY

P. PARIJA

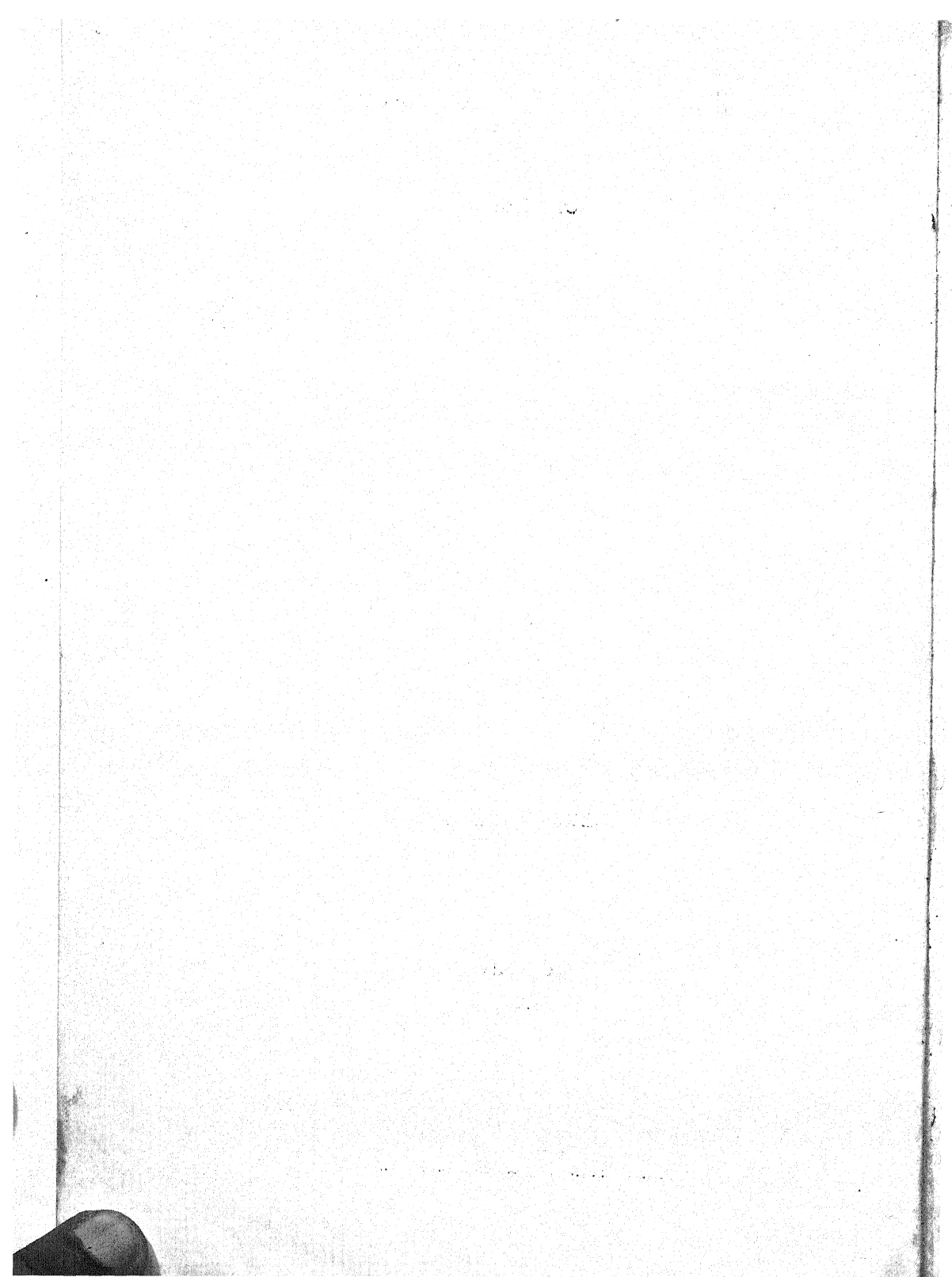


Vol. XIV

1935

PRINTED BY

ASSOCIATED PRINTERS, MOUNT ROAD, MADRAS



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The Journal of the Indian Botanical Society

(Formerly "The Journal of Indian Botany")

VOL. XIV

MARCH, 1935

No. 1

LIGHT AND FUNDAMENTAL LIFE PROCESSES OF PLANTS

*Presidential Address, Annual Meeting of the Indian Botanical
Society, January, 1935*

BY

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Introduction

The phenomena of light are mainly studied by physicists, but the knowledge derived from their labours about the nature of light is of great importance to those who study the life processes of plants. If we go to the very root of things it becomes abundantly clear that there can be no life without light and the sensation that we know as light can only be experienced through the medium of life. Light is known to be the carrier and supplier of energy without which life cannot exist. It is therefore natural that the recent advances in the study of the physical nature of light have been of great interest to plant physiologists and are paving the way to the right understanding of the various processes taking place in plants under the influence of this all important phenomenon of Nature.

Plants by virtue of their unique activities entrap the energy that they receive from the sun's rays, work it up in a mysterious manner and produce the complex organic substances which not only maintain them in the state of living, but also maintain the multifarious forms of the animal life in the living state on the earth. The energy of the sun's rays that is harnessed and stored up in these products of plant manufacture is liberated again in an equally mysterious manner and becomes available for the important manifestations of the living organisms. Thus the living is made possible by the energy that is primarily derived from sunlight through the agency of plants whose strength, to use the quaint saying, was supposed to lie in standing still.

The sensation of light firstly makes us conscious of the entire Universe and all that it consists of. It has made possible the stock of human knowledge and experience that we now so proudly possess and that we so rapidly enlarge. Secondly the energy of light is the mainstay of living organisms. We are here more concerned with the second than with the first proposition. The first is the outcome of the second. It is therefore of paramount importance to direct our efforts to the study of these beneficent processes in plants that make the living possible. The knowledge of these processes will benefit mankind more than any other discovery in science and any small attempt to add a few facts to the existing stock of knowledge about these processes will be an attempt in the service of mankind. It would be therefore in my opinion a fitting occasion to speak on the present state of knowledge of the relations of light to these processes by which complex-organic food substances are manufactured, before this society which has, as its aim, the promotion of research in different fields of the plant sciences.

Light and Synthesis of Carbohydrates

The importance of light on synthesis of carbohydrates in leaves was first noticed by Ingen-Housz (1779). Senebier (1788) next observed the effect of light of different colours in the formation of starch in leaves. This work was extended by Gilby (1821), Daubeny (1836) and Dumas and Boussingault (1841). Draper (1844) next pointed out that the yellow region of the visible spectrum was the most effective but later Lommel (1871) showed that the photosynthetic activity was highest in the red yellow region. Timmiazeff (1869-89) confirmed the findings of Lommel and he (1890) showed that the formation of starch increased from the red end of the spectrum towards the violet end. Englemann (1882-1884) on the other hand found by his bacteria method a secondary maximum in the rate of the process in the blue violet region. In all these experiments no attempt was made to keep the energy contents of the different rays of light the same. This defect was

removed by Kniep and Minder (1909) in their experiments. These authors concluded that the rate of photosynthesis was the same in the red (620 $\mu\mu$ to infra red) and the blue (523-340 $\mu\mu$) regions and the process was at a standstill in the green region (524-512 $\mu\mu$). Though they made the intensities of the different rays equal in these experiments they used very low light intensity which acted as a limiting factor. They also employed an inaccurate method for the measurement of photosynthesis.

Ursprung (1912) further showed that the process of photosynthesis occurred only in the visible region of the spectrum of white light, the maximum being in the red region. The work of Ursprung (1917-18) fixed with certain degree of accuracy the exact spectral limits of photosynthesis, though they varied in the case of different plants. The photosynthetic activity was also found by Lubimenko (1923) to be more intense in the red region (760-600 $\mu\mu$) than in the blue region (480-400 $\mu\mu$) of white light. The discovery of this fact has stimulated research on the effect of different wavelengths of light on photosynthesis. The difficulties of experimentation on this problem are so great and in some ways so insurmountable that experimental work done by different workers on this aspect of the problem is defective for one reason or another. In order to obtain reliable experimental data it is necessary to make the total intensity of incident rays equal in all cases. Even when that is done, it is not certain if the different rays like the red and the blue are absorbed by a leaf to the same extent. If that is not the case the rates of photosynthesis in the two parts of the visible spectrum cannot be compared.

Light of Different Wavelengths and Photosynthesis

Attempt was made by Wurmser (1920-21) to determine the general relations between the quantity of energy absorbed from lights of different wavelengths and the rate of photosynthesis and came to the conclusion that green light was utilised in the photosynthetic work to about four times the extent of the red. To express it in physical terms the utilisation factor, *i.e.*, the ratio of the quantity of the light energy absorbed to the amount which is transformed into chemical energy, increases from red to green from 60 per cent. to 70 per cent. The main objection to the results of Wurmser (1920-21) is that the method of calculating the amount of absorbed energy has no direct physical basis. This difficulty is met by Warburg and Negelein (1923) by using a silvered vessel for the assimilating material so that the incident light energy is taken as the energy absorbed. They found that the efficiency of the photosynthetic system decreases with decreasing wavelength. Thus the utilisation factor shows decrease from 60 per cent. to 40 per cent. which is in accordance with Einsteins's Law of Photochemical Equivalence. In these experiments by Warburg and Negelein in the

errors in determinations of the energy absorbed by the assimilating organism are not removed. It is necessary to determine the fraction of light energy that is absorbed by the green pigments alone. Some energy is also absorbed by the colourless components of the tissues. Briggs (1929) tries to avoid the sources of error in measuring the absorbed amount of energy by an indirect method from the volume of oxygen evolved in photosynthesis, the energy utilised in the process is calculated from the heat of combustion of glucose to produce one cc. of carbon dioxide which is taken as equal to oxygen evolved. His results also confirm the findings of Warburg and Negelein (1923). In his experiments the energy incident on the leaves is not the same in the three regions, yellow, red, green and blue. The rates of photosynthesis are afterwards calculated for the same incident energy, *i.e.*, per 500 calories per 100 sq. cms. of the leaf area per hour. This is objectionable in the sense that the rate of photosynthesis in the different rays of light may not increase in the same proportion by the increase in their intensities. The incident light intensity employed by him is also very low.

The experiments done on the rate of reproduction in algae by Klugh (1925) also show that the rate of reproduction, *i.e.*, indirectly the rate of photosynthesis is highest in the red rays. Similar conclusions have been reached by Moore, Whiteley and Webster (1923) on the photosynthetic activity of the sea-weeds.

The main conclusion that can be drawn from the work quoted above is that the efficiency of the photosynthetic mechanism decreases with the decreasing wavelengths of light. All these costly and elaborate experiments do little more than confirm the findings of Senebier in 1788 with his simple technique of double-walled bell-jars containing coloured solutions.

The above conclusion does not however find support in the conclusions reached by Popp (1926) on the effect of the omission of the blue-violet and the violet regions of light on the growth of plants. The omission of these rays results in the greatly decreased production of carbohydrates in leaves. The results suggest that the blue-violet rays are important in the process in some way or the other.

Sunlight, Electric Light and Photosynthesis

Some fresh light on the question of the effect of different rays of white light on the photosynthetic activity is thrown by the experiments done in my laboratory on formation of carbohydrates in leaves exposed to sunlight and light from an electric lamp (Dastur and Samant, 1933).

The results clearly show that the photosynthetic process in green leaves does not proceed with the same speed in artificial light

like a gas-filled electric lamp as it proceeds in diffused sunlight of the same total intensity. The formation of carbohydrates takes place very slowly and in small quantities in leaves of plants exposed to artificial light as compared with the formation of carbohydrates in sunlight. The spectrum analysis of the lights from the two sources showed that, though the spectral composition was the same, the distribution of energy in the different parts of the visible spectra was not the same. The artificial light was more intense in the yellow-red region of the spectrum than the diffused sunlight; while the latter was more intense in the blue-violet region than the former. The distribution of energy is fairly uniform in the different parts of the visible spectrum of sunlight while it is not so in the visible spectrum of light from the electric lamp. These observations open up the question of the effect of different wave-lengths of light on photosynthesis. The artificial light is more intense in the yellow-red region of the visible spectrum (at a distance of 50 cms. at which the plants are exposed) than the diffused sunlight. If the efficiency of the photosynthetic system decreases with decreasing wavelengths of light, the results obtained by us do not support this conclusion, as the artificial light is richer in those rays which are supposed to be photosynthetically efficient. These results suggest that either the blue-violet region of the visible spectrum is equally or more efficient in the process than the yellow-red region or that the whole region of the visible spectrum is photosynthetically effective, and the lesser proportion of any one region results in a depressed rate of photosynthesis. The total energy supplied by the different radiations of the visible spectrum is not the determining factor in the process, but the different radiations as such or the frequency of radiations are important for the process. If it is merely a question of energy derived from light radiations, the process of photosynthesis should go on normally in artificial light supplying the same amount of energy in terms of ergs or calories, as supplied by the sunlight. These findings make us look at the problems of the energetics and mechanism of photosynthesis from a new angle and it was considered to be of interest to extend these observations.

Photosynthesis in Lights from Different Sources

In order to obtain further evidence to support the above conclusions it was undertaken to determine the rate of photosynthesis in leaves exposed to lights from different sources. Four different artificial sources, an electric lamp, daylight lamp, an incandescent oil lamp and a carbon arc lamp were used. The measurements of the distribution of energy in the visible spectrum of each light were made in three different ways namely, (1) by micro-thermopile, (2) by photographic plates and (3) by taking spectrum photographs by means of Adam Hilger's constant deviation spectrometer. According to the intensities of the blue-violet regions the sources of light are found to be in the following order:—(1) Sunlight,

(2) Carbon arc lamp, (3) Daylight lamp, (4) Electric lamp and (5) Incandescent oil lamp. The quantities of carbohydrates formed in leaves exposed to these five sources of illumination are also found to be in the same order. The quantities of carbohydrates formed in sunlight are higher than that formed in the carbon arc lamp. Similarly the carbohydrate contents of the leaves exposed to the arc lamp are higher than those of the leaves exposed to the daylight lamp. The carbohydrates formed in leaves exposed to the daylight lamp are significantly higher than the carbohydrates formed under the electric lamp and so on. As the total intensities are kept the same and as the main differences in the energy distribution in the different parts of the spectrum are mainly in the blue-violet region the only conclusion that can be drawn is that the blue-violet region must be playing an important part in the photosynthetic process and for normal photosynthetic activity both the red and blue-violet regions are equally important. If any one of the two regions is either absent or is of a very low intensity the normal photosynthetic activity does not proceed. (*Dastur and Mehta, in course of publication in the Annals of Botany.*)

Photosynthesis in the Red and Blue-Violet Lights

In order to put to test these conclusions, it was undertaken to measure the rate of photosynthesis in leaves exposed to monochromatic red and blue lights and to white light of equal intensities in the three cases. It was not found possible to use an artificial source of light for obtaining large beams of monochromatic red and blue-violet lights of sufficiently large intensity in order that light intensity may not act as a limiting factor. So the experiments had to be conducted in open sunlight. Fortunately this was possible during the dry months of the year. For obtaining monochromatic lights solution filters had to be used. After several failures a solution of carmine in lithium carbonate one centimeter thick was used for the red light and ammoniacal solution of copper sulphate 1 cm. thick was used for the blue-violet light. The range of transmission of the red filter was 7,000 to 6,200 \AA and that of the violet filter was 4,720 to 4,000 \AA . The spectrum photographs of the filters showed no transmission in any other part of the spectra. The percentage transmission of the different wavelengths in the transmitted red and blue-violet regions were determined. The maximum transmission in the red region was 46.77 per cent. at 6,800 \AA and in the blue-violet filters was 23.9 per cent at 4,200 \AA . By determining the total percentage transmission of the two filters it was possible to make the total intensities of the two rays equal. As the microthermopile is not equally sensitive to the red and blue rays, an indirect and complicated method was employed to make the intensities equal. The intensities of the red light and white sunlight were reduced and made equal to that of the blue-violet light by interposing glass plates.

The glass plates used do not interfere with the transmitted red region or the white light except lowering the total intensities. This is verified spectrometrically. The results obtained with the three lights show that the formation of carbohydrates is highest in leaves exposed to sunlight, medium in red light of equal intensity and least in blue-violet light of the same intensity. The differences in the quantities of total carbohydrates formed in the red, blue and white lights of equal intensities are statistically significant. (*Dastur and Mehta, in course of publication in the Annals of Botany.*)

If the results obtained with red and blue-violet lights are alone compared, they will appear in agreement with those obtained by previous workers. But if the results obtained with the white light are taken into consideration, they support the conclusion that the efficiency of the photosynthetic mechanism is highest in the full visible spectrum of light than in the monochromatic red and blue-violet lights. The importance of blue-violet region in the process is again proved by these experiments.

In view of the results obtained the question of the energetics of photosynthesis acquires fresh aspects. It appears the energy carried by the different radiations is not the only determining factor in the rate of the process. If that was the case there should not have been marked and significant differences in the carbohydrate contents of the leaves exposed to sunlight, arc lamp, daylight lamp, electric lamp and also the sunlight and the red and blue-violet lights, all of equal intensities. Therefore the differences found could only be attributed to the differences in the distribution of spectral energy. The blue-violet rays are as important in the process as the red rays and so the frequency of the different rays is another determining factor. It is probable that for the different stages in the process all rays of different wavelengths are used as it is likely that for the activation of the different reacting molecules the rays of different frequencies may be essential.

In the case of *Helianthus annuus*, L., calculations show that the volumes of carbon dioxide that must have been decomposed in the white, red and blue-violet lights are 89, 22 and 7 cc. respectively. In case of *Raphanus sativus*, L. the volume are 60, 32 and 9 cc. It is apparent that with the same amount of radiant energy supplied the efficiency of the photosynthetic mechanism in the red light is nearly three times that of the blue-violet light, while in the white light it is nearly four times its efficiency in the red light in *Helianthus annuus*, L. and is nearly double in *Raphanus sativus*, L. Thus the number of light quanta necessary for transforming a molecule of carbon dioxide increases in the white, red and blue lights as the utilisation factor decreases in the same order. Warburg and Negelein (1923) have estimated that the number of quanta absorbed per molecule of carbon dioxide reduced, should be four or five and they should remain constant at four for all wavelengths in the

visible spectrum. The results here show that that is not the case unless if it be assumed that the energy absorbed by the leaves is highest in the white light, medium in the red light and the least in the blue lights. But this assumption is not borne out by the results obtained with the different sources of light enumerated above.

Protein Synthesis in Lights from Different Sources

An interesting point arises from the results of carbohydrates formed in electric light and in sunlight. It is very likely that the lesser amounts of carbohydrates formed in leaves exposed to the electric lamp may be due to their rapid utilisation in protein synthesis, while in leaves illuminated by sunlight the formation of proteins may be taking place slowly leading to the accumulation of carbohydrates. If this supposition is found to be true it would again raise fresh issues on the effects of lights on protein synthesis. So far light is known to play an indirect rôle in the synthesis of proteins in as much as it is instrumental in the synthesis of the carbohydrates which are needed for the construction of proteins. Zaleski (1897-1901), Zaleski and Tutorski (1912), Stoklasa (1916), Muencher (1923), Pearsall and Loose (1933) have shown that the synthesis of proteins occurs four times as rapidly in sunlight as it occurs in absence of light. If that is the case, the lights of different spectral intensities may also bring about the differences in the rates of the synthesis of proteins. It was therefore necessary to determine the protein content of leaves of the same species exposed to sunlight, arc light, daylight lamp and electric lamp as were done in the case of carbohydrates. If the rate of photosynthesis is really depressed as the source of light is progressively poorer in the blue-violet rays, results of the total protein content of the leaves should also decrease in the same order.

The technique of experimentation employed is the same as before. The methods of extraction and determinations of protein nitrogen in different forms are carefully worked out and used. The leaves and petioles of plants exposed to these different lights of equal intensities are analysed for water-soluble protein nitrogen, polypeptide nitrogen, diamino nitrogen, monoamino nitrogen, amido nitrogen, acetone-soluble protein nitrogen, alcohol-soluble protein nitrogen, insoluble residual nitrogen and total nitrogen. The plants used were the same as before, *viz.*, *Ricinus communis*, L. *Helianthus annuus*, L. and *Abutilon asiaticum*, G. Don. Results obtained are particularly striking and interesting in many ways. In the leaves water-soluble protein and polypeptides and diamino acids are definitely produced during the exposure, while monoamino acids and amides do not show any increase after exposure. It may be that amides and monoamino acids are rapidly converted into soluble proteins and polypeptides just as hexoses are supposed to be rapidly converted into starch. The results of the total protein

contents of the leaves are in the order daylight bulb, arc light, electric light and sunlight, while the carbohydrate contents of the exposed leaves stand in the order sunlight, arc light, daylight bulb and electric lamp. If the carbohydrates supplied the basic materials for the formations of the proteins, the leaves exposed to sunlight should contain the largest quantity of proteins than those exposed to any of the three sources of light. If the supply of carbohydrates is not acting as a limiting factor the protein contents of the leaves should be equal in all the four lights. Why should there be an increased production of protein in the daylight bulb and arc lamp as compared to sunlight when the formation of carbohydrate is most rapid in the latter? It is evident that very small part of carbohydrates formed in the leaves by photosynthesis is utilised for protein synthesis as the protein content of the leaves exposed to electric light is slightly more than that of the leaves in sunlight, though the carbohydrates formed in the former is less than one half the amount formed in the latter. (*Dastur and Kanitkar, in course of publication*).

Conclusions

It is difficult to explain the differences in the synthesis of proteins in leaves exposed to different lights and still much more difficult it is to understand the low protein nitrogen contents of the leaves in sunlight where the photosynthetic activity is the highest. It may be possible that the machinery of the cells is clogged up owing to the very rapid production and consequent accumulation of the products of photosynthesis and hence the rate of synthesis of protein is depressed. In the case of electric light the protein synthesis goes on normally but its rate is inhibited by the depressed rate of photosynthesis, while in the daylight bulb and under arc lamp there is an increased production of proteins due to the increased rates of photosynthesis which however are not rapid enough to clog up the active cells. It is, however, difficult to understand why the protein synthetic activity should be inhibited in sunlight and the photosynthetic activity should continue unabated even after the cells are full of starch. It is also premature to say that the synthesis of protein is influenced by the differences in the spectral intensities like the synthesis of carbohydrates shown above.

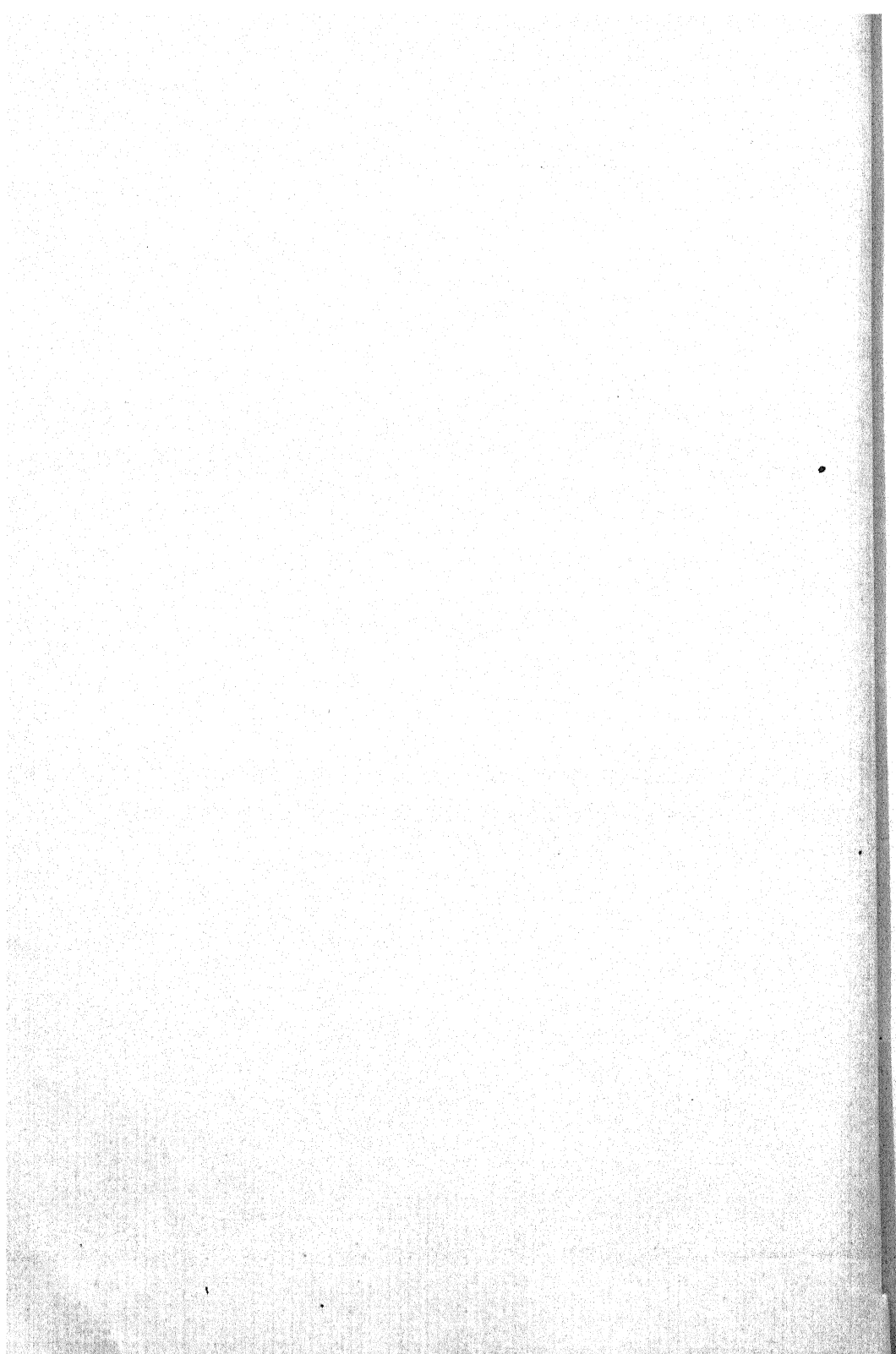
It thus appears that the rays of different wavelengths of the visible part of the spectrum of white light are as important for the two fundamental constructive processes in plants as their energy contents. These results have added to our difficulties in understanding the energetics of the mechanism by showing that the respective intensities of the different radiations of white light are as important as their total energy contents. If the above findings are true, the synthetic processes of the plants are greatly influenced by variations in light intensities that normally occur during the day and season

of the year. The growth of plants is the net result of the constructive and destructive processes and if the constructive processes are so affected by the diurnal or seasonal changes in the quality and the quantity of light, it is no wonder that the reproductive activities of the crop plants show such wide variations.

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THELEPHORACEÆ OF BENGAL—I

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(Received for publication on 21st October 1933)

Introduction

The *Thelephoraceæ* is a group of Hard Fungi which is of great economic importance as its members are responsible for the decay of wood, timber, fence-posts, railroad sleepers, etc., causing great loss and finally rendering them practically worthless. This group is characterised in having the hymenium inferior or amphigenous, spread over smooth, rugose, rarely ribbed or papillate surface, coriaceous or waxy in nature and having an intermediate layer of hyphæ lying between the hymenial layer and the mycelium. And it constitutes a small assemblage of fungi. But there exists a great diversity of opinion as to whether this group represents an order, a family or a tribe.

In this paper detailed studies of *twenty-four* species belonging to *four* genera, *viz.*, *Stereum*, *Hymenochaete*, *Craterellus* and *Asterostromella* have been made with special reference to their anatomy.

The author has followed Burt's (1) classification of the *Thelephoraceæ* throughout with such modifications as have been found necessary. In working out the anatomy of the individual species the works of Burt (2), Overholts (3) and Corner (4-5) have been of great help.

Three different systems of hyphæ, *viz.*, skeletal, generative and binding, have been found involved in the construction of the fruit-bodies of the *Thelephoraceæ* in the Bengal species so far studied by the author. He has followed Corner (5) in using the terms *dimitic*, *i.e.*, composed of two systems of hyphæ, *trimitic*, with three systems,

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and *monomitic* with only one system of hyphæ. Though in other countries intensive study of Hard fungi has been made, unfortunately very little attention has been paid to it in India, specially in Bengal from where only *nine* species have so far been reported (6).

It is intended to bring out a systematic account of the family as represented in Bengal and this forms the first paper of the series. The material has been mostly collected from Calcutta and suburbs during the years 1930-32 in the months of July to November when they are very common.

All the species of Bengal *Thelephoraceæ* are saprophytic. The stipitate and merismatoid species grow sometimes on dead wood and sometimes on ground. When on ground they grow near the base of the tree or in the neighbourhood of half-buried wood, sometimes at the junction of the log and the soil. The effuso-reflexed species grow on dead wood causing its decay. None of the species however has been found to be parasitic in nature.

The author takes this opportunity of expressing his indebtedness to Prof. S. P. Agharkar for his invaluable advice and kindness in affording facilities in various ways during the earlier part of the investigations; to Prof. S. R. Bose, for his interest in the work and valued suggestions and for his generous help with some of his collections and also to Dr. H. Chaudhuri of the Punjab University, for the kindly interest he has taken in the work. The author also wishes here to express his great appreciation of the promptness with which Prof. E. A. Burt of the Missouri Botanic Gardens confirmed many of the determination of the species.

Methods and Technique

The technique employed by the writer for systematic study of the *Thelephoraceæ* has been divided into two main divisions, *viz.*, (1) Methods for the study of macroscopic characters, and (2) those for the study of microscopic characters.

1. *Methods for the study of microscopic characters.*

All the specimens have been examined either with a pocket lens or with the unaided eye. Colour of the fresh and dried specimens, which is an important character, has been noted in terms of the "Colour standard and colour nomenclature" of Ridgway.

2. *Methods for the study of microscopic characters.*

Methods employed here have been discussed under the following heads:—(a) Sectioning, (b) Staining and mounting, (c) Examination of the hyphal composition of the fruit-body, and (d) Spore-prints.

(a) *Sectioning*.—In making microscopical preparations all sections have been cut by free-hand sectioning method, mostly from fresh materials. When dried specimens were used the usual procedure followed by Burt (7) and Overholts (8) has been followed and this has proved very satisfactory. The brief outline of the process is as follows:—A piece of the material to be sectioned is placed in a high grade of alcohol for 1 to 2 minutes, and then transferred to water and allowed to remain for sometime until soft and then cut in elder pith. While sectioning 95 per cent. alcohol is used on the razor and afterwards the sections are transferred to water. At the time of examination of the sections a preliminary treatment with 7 per cent to 10 per cent. KOH solution as recommended by Burt is made and all measurements are taken at this stage. Overholts highly recommends this method and observes that "sections cut and mounted in this way show the fungus tissue in a remarkable state of turgidity comparable to that of fresh material." Close observations of the sections have to be made at this stage for in many species this alkaline solution dissolves out the colour of the hyphæ. Sections of the species with brown context, specially of the *Hymenochaete* and a few species of *Stereum*, turn dark on coming in contact with KOH solution. In this case lactic acid or lactor-phenol has been successfully substituted for KOH solution. Sections were always cut parallel to the direction of hyphæ in the fruit-body.

(b) *Staining and mounting*.—Several stains, such as Eosin, Magdala red, Safranin T, and Cotton blue were tried. For temporary mounts 1 per cent aqueous eosin and lactic-cotton-blue gave good results. Sections stained in lactic-cotton-blue and afterwards mounted in Lacto-phenol were found to be quite satisfactory for preliminary observations. It gave best results with very thin sections or with sections in which hyphæ are loosely arranged. In mounting sections of species with dark context, the lacto-phenol-cotton-blue (9) has been found to be very satisfactory. It seems to be a superior stain for mycological work. For making permanent preparations the writer followed the methods employed by Overholts (10) which are essentially those of Burt with slight modifications. The sections are treated with 5 per cent. to 10 per cent. KOH solution for a few

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minutes and then stained with 10 per cent. aqueous eosin solution for a few minutes till the sections are slightly overstained. These are then transferred directly to a few drops of acidulated glycerine and 10 per cent. acetic acid added immediately to fix the stain. After the acidification is complete the sections are mounted in 50 per cent. glycerine and set aside to allow the water of the glycerine to slowly evaporate. When the desired concentration is reached, more pure glycerine is run under the cover-slip and sealed with gold-size or with bee's wax mixed with venetian turpentine or canada balsam.

Sections of the species with brown context are stained satisfactorily with lactic-cotton-blue and mounted either in lacto-phenol or in lactic-acid.

(c) *Examination of the hyphal composition of the Fruit-body.*—In studying the hyphal composition of the fruit-body, thick sections of fresh materials were teased well with very fine mounted needles under a dissecting microscope. Sections from dried herbarium material were also teased and studied in this way. Best results were obtained, in cases other than the species of *Hymenochæte*, after a preliminary over-night treatment of the sections with 10 per cent. KOH solution. This hastens the process and the hyphæ at this stage are in a remarkably turgid condition. Sections of *Hymenochæte* and of those that are affected with KOH solution are best teased either in lacto-phenol or in lactic-acid slightly heated over a flame. In order to study the generative hyphæ showing clamp-connections, observations were made with the aid of 1/12th oil-immersion objective. Crushed mounts of the tissue of the fruit-bodies in glycerine-alcohol have also been found convenient for recognising the different types of hyphæ, and also for their measurements, but the difficulty lies in the fact that the hyphæ are broken into small bits so that one cannot trace their lengths to a considerable distance. The former method was very suitable for this purpose. Staining seems to be unnecessary, for the hyphæ are mostly coloured and when they are mounted in lacto-phenol and the sub-stage diaphragm and the condenser are slightly regulated, their visibility is decidedly increased.

(d) *Spore-prints.*—Spore collections from fresh materials and the record of the colour of spores are of immense importance for the accurate determination of species, for spores obtained from herbarium material are often hyaline and also shrinkage occurs in them. For this reason spore-prints have been made for most of the species and the method employed for the purpose is as follows:—

Fresh mature specimens were kept in a moist condition over clean glass slides with the hymenial surface downwards. The falling spores adhere to the glass surface and were protected from dust by enclosure in a tightly fitting cover. At this stage the colour was noted and measurements were made.

The following table shows the genera and species of Bengal *Thelephoraceæ* described in this paper:—

I. *Hymenochaete*.

H. aspera, *H. tenuissima*, *H. nigricans*, *H. rubiginosa*, *H. cacao*,

II. *Stereum*.

1. Central-stemmed species:—*S. nitidulum*, *S. elegans*.
2. Lateral-stemmed species:—*S. glabrescens*, *S. crenatum*.
3. Merismatoid species:—*S. petalodes*.
4. Effuso-reflexed species:—*S. fuscum*, *S. papyrinum*, *S. percome*, *S. umbrinum*, *S. endocrocinum*, *S. schomburgkii*, *S. vibrans*, *S. scytale*, *S. alternum*, *S. annosum*, *S. hirsutum*, *S. fasciatum*.

III. *Asterostromella*:—*A. rhodospora*.

IV. *Craterellus*:—*C. cornucopoides*.

Descriptions

Hymenochaete Lévillé. Plate I, Figs. 1—10.

Fructifications coriaceous to hard, very variable in form, stipitate or sessile, dimidiate, effuso-reflexed or resupinate, often densely imbricate. Hymenium inferior, even to setulose, velvety, rarely granular, characterised in having slender, conical, coloured setæ between the basidia; basidia tetrasporous. Spores white, smooth. Perennial, growing on wood.

Sections of all the species of *Hymenochaete* darken when treated with dilute (7-10 per cent.) KOH solution which turns the sections opaque.

This well-marked genus was first erected by Lévillé characterised by the presence of smooth, acute, thick-walled, coloured setæ in the hymenium. Lloyd did not accept it as a separate genus and included it as a section of his genus *Stereum*. But is of opinion that *Hymenochaete* is a genus of tropical species rather than of the cooler portion of north temperate zone. According to him *Hymenochaete* attains its greatest development both in form and in number of species in the western continent.

1. *Hymenochaete aspera* Berkeley and Curtis. Plate I, Figs. 1 & 2.

Distribution and Habitat.—India. Collected and reported for the first time from Bengal, Calcutta and also Cuba, Jamaica and Venezuela. Rare. Collected in October, 1930. Growing on a dead log.

Fructification.—Dimidiate, sometimes wedge-shaped, with a narrow base, imbricate, thin, pliant when dry so that it may be folded without breaking, laterally confluent, about 2.5 to 5 cm. long, about 2.5 to 4 cm. broad and about .5 mm. thick; margin fimbriate.

Upper surface.—Rough, covered with coarse, strigose, matted hairs; concentrically zoned, colour snuff-brown or Argus brown, sometimes becoming blackish-brown near the base.

Flesh.—Thin, leathery, colour paler than that of the upper surface.

Hymenial surface.—Uneven, irregularly tuberculose, with raised ridges, colour snuff-brown or ferruginous brown, in old specimens isabella-colour.

Spores.—Hyaline, smooth, globose, dimension about $3\text{--}4\ \mu$ or sub-globose, dimension about $3 \times 4\ \mu$.

Basidia.—Simple, hyaline, clavate, dimension about $14\text{--}18 \times 6\text{--}8\ \mu$.

Setæ.—Dark brown, conical, with a sharp pointed hayline apex, a narrow lumen extending from base to apex, frequent, arising mostly from the hymenium, a few from the sub-hymenium, dimension about $22\cdot5\text{--}49\cdot5 \times 9\text{--}11\ \mu$ and emerging upto $13\cdot5\text{--}31\cdot5\ \mu$.

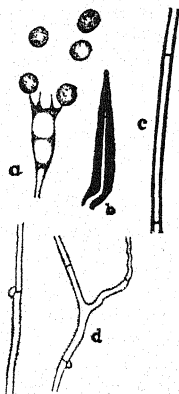


Fig. 1. a. Basidium and spores, $\times 750$; b. Setæ, $\times 375$; c. Skeletal hyphæ, $\times 375$; d. Generative hyphæ, $\times 750$.

Tissue differentiation.—Three distinct layers can be recognised and they are (a) a narrow setigerous layer, about $50\ \mu$ thick, along with the hymenial layer, (b) an intermediate layer of $70\text{--}78\ \mu$ thick, composed of longitudinally arranged sub-parallel hyphæ, and (c) the outermost hairy covering, about $210\text{--}280\ \mu$ thick, composed of interwoven hyphæ.

Hyphal systems.—The fruit-body is of *dimitic* construction. The systems of hyphæ are:—(a) Skeletal hyphæ:—brown, thick-walled, straight or slightly flexuous, septate, unbranched, about $2\cdot5\text{--}4\ \mu$ thick, wall-thickness about $\cdot5\ \mu$ and (b) Generative hyphæ:—Thin walled, hyaline, septate, closely or sparingly branched, densely granular, about $2\ \mu$ thick, a few clamps were recognised.

2. *Hymenochaete tenuissima* Berkeley. Plate I, Figs. 3 & 4.

=*Stereum tenuissimum* Berk.

According to Bresadola *Hymenochaete tenuissima* Berk. is identical with *Hymenochaete rheicolor* Mont. (Bresadola, Ann. Myc., 14 : 233, 1916).

Distribution and Habitat.—India, Ceylon, the Himalayas, now collected and reported from Bengal, Calcutta, Darjeeling; and also South Carolina, West Indies, Mexico and S. America to Paraguay and Chile and Australia. Rare. Collected in October, 1931. Growing on dead wood.

Fructification.—Thin, papery, flexible when dry so that it may be folded without breaking, generally producing orbicular pilei, centrally attached, sometimes imbricate, often laterally confluent, or effuso-reflexed and spreading along a branch in a continuous sheet and producing flabelliform pilei at the margin; the resupinate part easily separable from the substratum, reflexed pilei about 15-17 mm. long, about 15-32 mm. broad, and about .25—1.5 mm. thick; margin lobed.

Upper surface.—With distinct concentric zones, dull, usually clothed with coarse pubescence collected into small fascicles; usually matted strigose without radial fascicles, colour at first gilvous becoming antique brown and finally Argus-brown.

Flesh.—Membranaceous, flexible.

Hymenial surface.—Uneven, radiately rugose, colour Isabelline to Dresden brown.

Spores.—Hyaline, smooth, sub-globose, dimension about $2.5-3\ \mu$ to elliptic, dimension about $3.4 \times 2.2-2.5\ \mu$.

Basidia.—Simple, hyaline, cylindrical, dimension about $10-14 \times 4.5\ \mu$.

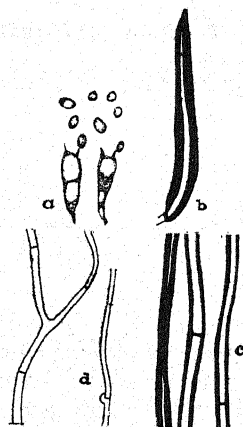


Fig. 2. a. Basidia and spores, $\times 750$, b. Setæ; c. Skeletal hyphæ; d. Generative hyphæ, $\times 375$.

Setæ.—Dark red-brown, conical, acute, with a narrow central lumen extending throughout or part of its entire length, frequent, arising from the sub-hymenium, dimension about $40-75 \times 8-10\ \mu$,

emerging beyond the basidial layer upto 38-60 μ . (Plate IX, Fig. 48.)

Tissue differentiation.—The tissues are differentiated into three distinct layers as follows:—(a) a setigerous layer along with the hymenial layer, about 42-75 μ thick, (b) an intermediate layer about 70-112 μ thick, composed of thick-walled longitudinally arranged sub-parallel hyphæ, and (c) the outermost hairy covering upto 1,120 μ thick.

Hyphal systems.—The fruit-body is of *dimitic* construction.

The different systems are:—(a) Skeletal hyphæ:—brown, thick-walled, unbranched, septate, straight or slightly flexuous, longitudinal, about 2.25-6.75 μ wide, wall-thickness about 1-2 μ , lumen, more or less obliterated in mature parts, and (b) Generative hyphæ:—Thin-walled, hyaline, sparingly branched, septate, interwoven or longitudinal, with densely granular protoplasm, about 1.4-5 μ wide, clamps present, few.

3. *Hymenochaete rubiginosa* Dickson ex Léveillé.

Plate I, Figs. 5 & 6.

- = *Helvella rubiginosa* Dickson,
- = *Thelephora rubiginosa* Schrader,
- = *Stereum rbriginosum* Fries,
- = *Auricularia ferruginea* Bulliard,
- = *Stereum ferrugineum* Bulliard ex Fires,
- = *Hymenochaete ferruginea* (Bulliard) Massee,

Distribution and Habitat:—India.—The Nilgiris, now collected and reported from Bengal — Calcutta, Behala and Ballygunj; and also Britain, Europe, Canada to Mexico, westward to Oregon and California, and in Porto Rico; Cuba, Patagonia, Ohio, Sierra Nevada, Rhodes, Borneo, Australia, Tasmania and Bonin islands. Formerly *Hymenochaete rubiginosa* was recorded as occurring in India, but no definite reference was noted. "No Indian specimens were found in the collections of Léveillé at Paris" — Butler. Rare. Collected in August to November 1930-32. Growing on dead wood, logs, etc.

Fructification.—Reflexed, spread out, sometimes becoming resupinate, separable, densely imbricate, about 1.5-3 cm. long, about 2-3.5 cm. broad, and about .5 mm. thick; margin entire, thin, colour ochraceous tawny.

Upper surface.—Marked with narrow, concentric zones, velvety, colour rubiginous or Brussel's brown, becoming smooth and date-brown or fuscus black with age.

Flesh.—Leathery, rigid, colour tawny ferruginous.

Hymenial surface.—More or less colliculose, bristly (setulose) under a lens, faintly zoned, colour ferruginous or bistre.

Spores.—Oval, hyaline, smooth, dimension about $4.5 \times 3 \mu$.

Basidia.—Clavate, hyaline, dimension about $16-20 \times 4.5 \mu$.

Setæ.—Dark brown, conical, with a narrow lumen in the middle, apex sharply pointed, very numerous, arising from all parts of the setigerous layer, dimension about $31.5-55 \times 4.5-9 \mu$, emerging upto $13.5-27 \mu$.

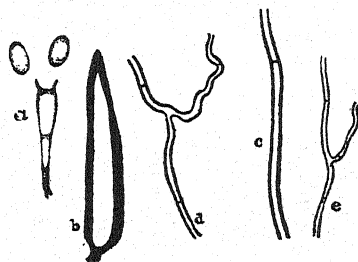


Fig. 3. *a*. Basidium and spores, $\times 750$; *b*. Setæ, $\times 375$; *c*. Skeletal hyphæ, $\times 375$; *d*. Binding hyphæ, $\times 375$; *e*. Thin-walled hyphæ, $\times 750$.

Tissue differentiation.—Four distinct layers can be recognised and they are:—(*a*) a very broad setigerous layer of $70-148 \mu$ thick along with the hymenial layer, (*b*) an intermediate layer of 210μ thick composed of longitudinally arranged interlaced hyphæ, (*c*) a dense dark zone about $28-42 \mu$ thick, composed of longitudinally arranged sub-parallel hyphæ, and (*d*) the uppermost hairy covering of $70-140 \mu$ thick, composed of matted, interwoven hyphæ.

Hyphal systems.—The fruit-body is composed of three systems of hyphæ and they are:—(*a*) Skeletal hyphæ:—Thick-walled, brown, straight or slightly flexuous, unbranched, septate, about $2-4 \mu$ thick, wall-thickness about $.25-1 \mu$, (*b*) Binding hyphæ:—Thick-walled, brown, much branched, flexuous, interwoven, septate, about $1.5-2 \mu$ thick, wall-thickness about $.25-.5 \mu$, (*c*) Thin-walled, hyaline hyphæ, densely granular, sparingly branched septate, without any clamp, about $.5-1 \mu$ thick.

4. *Hymenochaete nigricans* Lévillé ex Bresadola.

Plate I, Figs. 7 & 8.

= *Stereum villosum* Lév.

= *Hymenochaete strigosa* B. & Br.

= *Hymenochaete spadicea* B. & Br.

= *Hymenochaete phœa* Berk.

Distribution and Habitat.—India, Ceylon, Bombay (Blatter), Khandala, now collected and reported from Bengal, Calcutta and

Darjeeling Districts; and also Malacca, Java, Australia and New Zealand. Rare. Collected in August-October, 1930-31. Growing on logs, posts, etc.

Fructification.—Thin coriaceous, flexible when dry, effuso-reflexed, often imbricate, resupinate areas spreading along a branch and producing flabelliform pilei at the margin, reflexed portion about 10-15 mm. long, about 15-30 mm. broad and about .25 mm. thick; margin undulated, thin.

Upper surface.—Velvety, dull, clothed with coarse, matted pubescence generally not collected into fascicles, rarely faintly zoned near the base, colour Argus-brown and deep chestnut-brown.

Flesh.—Membranaceous, flexible.

Hymenial surface.—Uneven, with fine radial striations, with the depressed zones of the pileus showing on its surface, colour snuff-brown or Isabelline.

Spores.—Hyaline, smooth, sub-globose, dimension about $2.3\ \mu$ as seen on basidia.

Basidia.—Clavate, hyaline, dimension about $10-12 \times 6-8\ \mu$.

Setæ.—Dark brown, cylindrical from base, abruptly tapering to a hyaline sharp pointed apex; apex sometimes curved, with a narrow lumen in the middle extending to some part of its entire length, frequent, arising mostly from the hymenium, dimension about $22.5-54 \times 9-13.5\ \mu$ emerging beyond the basidial layer upto $9-27\ \mu$. (Plate IX, Fig. 53.)

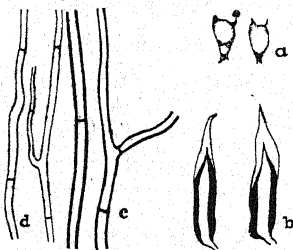


Fig. 4. a. Basidia and spores; b. Setæ; c. Skeletal hyphæ; d. Thin-walled hyphæ, $\times 375$.

Tissue differentiation.—Three differentiated layers are recognised as follows:—(a) a setigerous layer along with the hymenial layers, about $54\ \mu$ thick, mixed with erect, interlaced hyphæ, (b) an intermediate layer of longitudinally arranged sub-parallel hyphæ, about $56-70\ \mu$ thick, and (c) the outermost hairy covering of $280-420\ \mu$ thick composed of thick-walled interwoven hyphæ.

Hyphal systems.—The fruit-body is of *dimitic* construction. The systems are:—(a) Skeletal hyphæ:—brown, thick-walled, straight or flexuous, sparingly branched, septate, about $2.5-3\ \mu$ wide, wall-thickness about $.5\ \mu$ and (b) very thin-walled, hyaline, hyphæ, with densely granular protoplasm, septate, richly or sparingly branched, without any clamp-connection, about $1.5-2\ \mu$ wide.

5. *Hymenochaete Cacao* Berkeley. Plate I, Figs. 9 & 10.

= *Stereum Cacao* Berkeley.

Distribution and Habitat.—India:—Khasia Mts. (J. D. Hooker); now collected and reported from Bengal, Calcutta; also Jamaica, Cuba and Venezuela. Rare. Collected in October 1931. Growing on stumps of trees.

Fructification.—Sessile, closely imbricated and connate, thin but somewhat rigid, flabelliform, sometimes partly resupinate forming more or less orbicular patches, deeply lobed and plicate, about 2 to 3 cm. long, about 1.5 to 2 cm. broad and about .5 mm. thick; margin lobed.

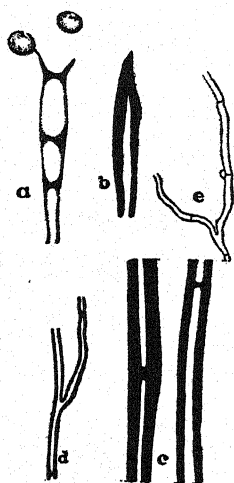


Fig. 5. a. Basidium and spores, $\times 750$; b. Setæ, $\times 750$; c. Skeletal hyphæ, $\times 750$; d. Binding hyphæ, $\times 375$; e. Generative hyphæ, $\times 375$.

Upper surface.—Velvety, becoming smooth with age, furrowed with a few narrow concentric zones, colour Brussels-brown.

Flesh.—Thin, colour same as that of the upper surface, rigid, leathery.

Hymenial surface.—Uneven, with a few concentric ridges, colour between fuscus and blackish-brown (or tobacco-brown).

Spores.—Hyaline, even, sub-globose, dimension about $3\text{--}4 \times 2\text{--}3 \mu$.

Basidia.—Clavate, hyaline, dimension about $20\text{--}26 \times 3\text{--}4 \mu$.

Setæ.—Dark brown, cylindrical, abruptly tapering to a very sharp point, lumen narrow, very numerous, arising from all parts of the setigerous layers, dimension about $18\text{--}22\text{--}31 \times 4\text{--}5\text{--}6\text{--}5 \mu$ emerging beyond the basidial layer upto $9\text{--}18 \mu$. (Plate IX, Fig. 46.)

Tissue differentiation.—Three distinct layers are recognisable and they are:—(a) a setigerous layer of 42μ thick along with the hymenial layer, (b) an intermediate layer of $140\text{--}210 \mu$ thick, composed of longitudinally arranged sub-parallel hyphæ, bending on one side into the hymenial layer, and (c) the outermost hairy covering composed of interwoven sub-erect hyphæ, about $70\text{--}100 \mu$ thick.

Hyphal systems.—The fruit-body is of *trimitic* construction. The different systems of hyphæ are:—(a) Skeletal hyphæ:—Brown, thick-walled straight, unbranched, septate, about $3\text{--}6 \mu$ thick, wall-thickness about $1\text{--}2\text{--}5 \mu$, (b) Binding hyphæ:—Brown, thick-walled, interwoven, flexuous, sparingly branched, about $3\text{--}4 \mu$ thick, wall-thickness about $1\text{--}1\text{--}5 \mu$ and (c) Generative hyphæ:—Thin-walled, hyaline, interwoven, densely granular, richly or sparingly branched, septate, about $2\text{--}3 \mu$ wide; a few clamps present.

Stereum Persoon. Plates II—VIII, Figs. 11—44.

Fructifications coriaceous to hard, very variable in form, pileate, stipitate or sessile, infundibuliform, dimidiate, resupinate or effuso-reflexed. Stem central or lateral. Hymenium inferior without any setæ, smooth, sometimes pubescent or velvety, rarely rugulose or ribbed; normally an intermediate layer of longitudinally arranged hyphæ present. Flesh pale. Basidia simple. Spores white, generally smooth, rarely granular. Annual or perennial, growing on wood or on ground.

1. *Stereum elegans* Fries. Plate II, Fig. 11.

= *Thelephora elegans* Meyer.

Distribution and Habitat.—India:—Ceylon, Bombay, Malabar, Lower Burma, Saharanpur, Khandala; now collected and reported from Bengal, Behala, Howrah district and Burdwan district; also Brazil, St. Domingo, Cuba, Venezuela, Tasmania, Australia, New Zealand, Africa. Common. Collected in July and August 1930. Growing in rosettes usually on ground near the base of the tree or in the neighbourhood of half-buried wood, sometimes at the junction of the log and the soil.

Fructification.—Stalked, densely cæspitose, separately stalked, but confluent above, imbricate, usually infundibuliform, sometimes

split on one side, often flabelliform or spatulate, coriaceous, flexible, about 2.5 cm. high, pilei 1.2-5 cm. in diameter, less than .5 mm. thick; margin lobed, thin, sometimes split up when dry.

Upper surface.—Glabrous, colour in young specimens when fresh, bright, pale-yellow to ochraceous with yellow-brown to purple-brown concentric zones, becoming deep red-brown and coarsely ridged when dry, sometimes older specimens drying almost black, not zoned, glabrous, and with fine radial ridges.

Flesh.—Thin, white, coriaceous.

Hymenial surface.—Uneven due to folds, radially plicate, colour nearly white, isabelline when dry, pruinose.

Stem.—Central, solid, about .5 to 1.5 cm. long, about 2-3 mm. in diameter, stiff, branched above, colour when fresh, pallid, pale yellow or ochraceous covered with minute tomentum, becoming almost white or paler brown coloured and covered with rufous tomentum when dry; tomentum extending upto the base of the pileus.

Spores.—Hyaline, smooth, sub-globose, dimension about 3-4 μ .

Basidia.—Simple, hyaline, clavate, dimension about 18-20 \times 5-6 μ .

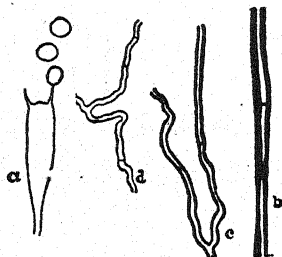


Fig. 6. *a*. Basidium and spores, $\times 750$; *b*. Skeletal hyphæ; *c*. Binding hyphæ; *d*. Thin-walled hyphæ, $\times 375$.

Tissue differentiation.—Two distinct layers are recognised as follows:—(*a*) the hyaline hymenial layer, about 20 μ thick, and (*b*) a compact hyphal layer, composed of densely interwoven hyphæ about 280-320 μ thick.

Hyphal systems.—The construction of the fruit-body is *trimitic*. The systems of hyphæ are:—(*a*) Skeletal hyphæ:—Thick-walled, hyaline, straight or flexuous, unbranched, distantly septate, about 4-5.5 μ wide, wall-thickness about .5-1 μ , (*b*) Binding hyphæ:—Thick-walled, hyaline, sparingly branched, very much flexuous, septate, about 2-4 μ wide, wall-thickness about .5-1 μ and (*c*) Thin-walled, hyaline hyphæ, flexuous, branched, septate, with dense protoplasmic contents, about 2-3 μ wide, without any clamp-connection.

2. *Stereum nitidulum* Berkeley. Plate II, Fig. 12.

Distribution and Habitat.—India:—Saharanpur, Kalsia, Poona, Ceylon, Himalayas, now collected and reported from Bengal, Behala, Shibpur, Hooghly, Howrah and Burdwan districts, also Brazil, Queensland, Australia, Cuba, and San Domingo. Common. Collected in July-September 1930-32. Growing on stumps and buried wood.

Fructification.—Stiptate, solitary or gregarious, separately stalked, not confluent above, usually infundibuliform, generally split and petaloid or minutely lineate when dry, coriaceous, flexible, about 2-5 cm. high, pilei about 5-3.5 cm. in diameter, less than .5 mm. thick; margin thin, generally toothed or lacinate.

Upper surface.—Glabrous, obscurely zonate, reddish bay or hazel.

Flesh.—Thin, coriaceous, pale.

Hymenial surface.—Glabrous, not zoned, even, pinkish buff when dry.

Stem.—Central, lateral-stemmed forms occurring fairly commonly, not covered with tomentum, rooting at base, solid, stiff, about 10-20 mm. long, about 1-3 mm. in diameter.

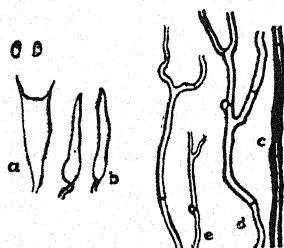


Fig. 7. *a*. Basidium and spores, $\times 750$; *b*. Gleocystidia, $\times 750$; *c*. Skeletal hyphae; *d*. Binding hyphae; *e*. Generative hyphae, $\times 375$.

Spores.—Hyaline, smooth, flattened on one side, dimension about $4-3 \mu$.

Basidia.—Clavate, hyaline, dimension about $16-18 \times 6 \mu$.

Cystidia.—Nil.

Gleocystidia.—Present, flexuous, clavate, arising from the subhymenium and extending into the hymenial layer, dimension about $20-36 \times 6 \mu$.

Tissue differentiation.—Two distinct layers have been differentiated as follows:—(*a*) a gleocystidial layer including the

hymenium, about 20-36 μ thick, and (b) a compact hyphal layer, about 280-350 μ thick, composed of longitudinally arranged sub-parallel hyphæ.

Hyphal systems.—The fruit-body is of trimitic construction. The different hyphal systems are:—(a) Skeletal hyphæ:—Thick-walled, hyaline, flexuous, distantly septate, unbranched, without any clamp-connection, about 3-4 μ wide, wall-thickness about 1-1.5 μ , (b) Binding hyphæ:—Thick-walled, hyaline, very much flexuous, interwoven, branched, septate, about 3-4 μ wide, wall-thickness about .5-1 μ and (c) Generative hyphæ:—Thin-walled, hyaline, much branched, flexuous, septate, with dense granular protoplasm, about 1-2 μ thick; clamp-connections very few.

3. *Stereum petalodes* Berkeley. Plate II, Figs. 13 & 14.

Distribution and Habitat.—Now collected and reported from India, Bengal — Ballygunj, Behala, Howrah and Burdwan districts; also San Domingo. Very common. Collected in July to September 1930-1932. Growing in rosettes on ground near the base of the tree or in the neighbourhood of half-buried wood, sometimes at the junction of the wood and the soil.

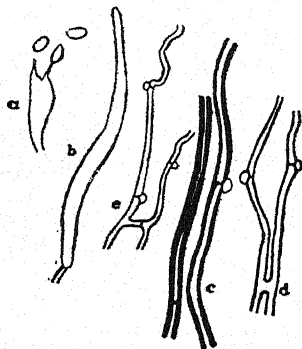


Fig. 8. a. Basidium and spores, $\times 750$; b. Gleocystidium, $\times 375$; c. Skeletal hyphæ; d. Binding hyphæ, e. Generative hyphæ, $\times 375$.

Fructification.—Sessile, coriaceous, flexible, faintly zoned, a rosette-shaped mass composed of many elongated pileate flaps, frequently laterally confluent by a broad base for several inches, at first infundibuliform, wedge-shaped or strap-shaped, soon split into numerous lacerate lobes which appear merismatoid and these again more or less sub-divided; margin thin, undulated, plicate.

Upper surface.—Uneven, marked with longitudinal grooves or striæ, colour reddish brown.

Flesh.—Thin, white, coriaceous.

Hymenial surface.—Uneven, much cracked, sometimes granular, colour dull, whitish.

Spores.—Hyaline, smooth, oval, dimension about $4 \times 2 \mu$.

Basidia.—Clavate, hyaline, dimension about $10-14 \times 4-6 \mu$ with 2-4 long sterigmata, about $4-5 \mu$ long.

Cystidia.—Nil.

Gleocystidia.—Present, hyaline, flexuous, cylindrical, frequent, arising from the sub-hymenium and gradually protruding into the basidial layer, dimension about $100-130 \times 6-10 \mu$.

Tissue differentiation.—Two distinct layers are differentiated as follows:—(a) a gleocystidial layer including the hymenium about 130μ thick, and (b) a hyphal layer, about $350-240 \mu$ thick, composed of densely interwoven hyphæ.

Hyphal systems.—The fruit-body is of *trimitic* construction. The different systems of hyphæ are:—(a) Skeletal hyphæ:—Thick-walled, hyaline, straight or curved, septate, unbranched, lumen almost obliterated in mature parts, without any clamp-connection, about $4-5 \mu$ wide, wall-thickness about $1-1.5 \mu$ (b) Binding hyphæ:—Thick-walled, hyaline, very much flexuous, interwoven, branched closely, septate about $2-4 \mu$ wide, wall-thickness about $.5-1 \mu$; both H — and clamp-connections present, and (c) Generative hyphæ:—Thin-walled hyaline, flexuous, much branched, closely septate, about $1.5-2 \mu$ wide, both H — and clamp-connection present.

4. *Stereum crenatum* Lévillé. Plate II, Fig. 15.

Distribution and Habitat.—Now collected and reported from India, Bengal, Howrah District; also Java and French Congo. Rare. Collected in July 1930. Growing on half-buried wood.

Fructification.—Stipitate, coriaceous, dimidiate, flabelliform, or wedge-shaped, narrowed towards the base into more or less distinct stem about 2-3 cm. high, about 3-4 cm. wide, and about 1-2 mm. thick; margin more or less lobed and irregular.

Upper surface.—Glabrous, faintly zoned, colour deep reddish-bay.

Flesh.—Coriaceous, pale, about 1-2 mm. thick.

Hymenial surface.—Even, white, sterile near the margin.

Stem.—Lateral, slender, rooting, minutely tomentose, colour whitish.

Spores.—Not found.

Basidia.—Not found.

Hyphal systems.—Two kinds of hyphæ can be recognised as follows:—(a) Thin-walled, hyaline, without any protoplasmic contents, longitudinal, closely septate, jointed, branched, about $8-10 \mu$

wide, and (b) thin-walled, hyaline, densely granular, septate, branched, interwoven, without any clamp-connection, hyphal-walls almost agglutinated, about 4-6 μ .

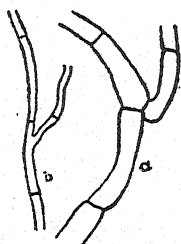


Fig. 9. a. & b. Thin-walled hyphæ, $\times 375$:

5. ***Stereum glabrescens*** Berkeley and Curtis.

Plate II, Figs. 16 & 17.

Distribution and Habitat.—Now collected and reported from India, Bengal, Howrah District; and also Cuba and West Indies. Rare. Collected in July 1930. Growing on fallen twigs.

Fructification.—Scattered, stipitate, sometimes two arising from a common mycelial pad, coriaceous, broad, flabelliform or spatulate, tapering to a distinct stem, about 7-20 mm. long, 5-10 mm. broad; margin thin, faintly wrinkled, pale, undulating.

Upper surface.—Minutely velvety to nearly glabrous, faintly zoned, colour verona brown to chestnut when dry.

Flesh.—Thin, coriaceous, pale.

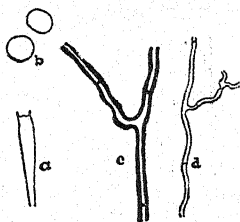


Fig. 10. a. Basidium, $\times 375$; b. Spores, $\times 750$; c. Skeletal hyphæ, $\times 375$; d. Thin-walled hyphæ, $\times 375$.

Hymenial surface.—Even, drying pinkish buff, paler towards the margin.

Stem.—Lateral, coriaceous, stiff, solid, about 10-16 mm. long.

Spores.—Hyaline, even, sub-globose, dimension about $5 \times 4 \mu$.

Basidia.—Simple, hyaline, cylindrical, dimension about $20-24 \times 6-7 \mu$.

Cystidia.—Nil.

Tissue differentiation.—The tissues are differentiated in a more or less stratified hyaline zone, about $70-84\ \mu$ thick, and a darker zone composed of densely interwoven hyphæ, about $42-140\ \mu$ thick.

Hyphal systems.—Two systems of hyphæ are recognised as follows:—(a) Skeletal hyphæ:—Thick-walled, hyaline, straight or slightly flexuous, septate, sparingly branched, about $3-6\ \mu$ wide, wall-thickness about $0.5-2\ \mu$ and (b) Thin-walled, hyaline hyphæ, flexuous, richly branched, septate, with dense granular protoplasm about $1-2\ \mu$ wide.

6. *Stereum fuscum* Schrader ex Quelet. Plate III, Figs. 18—23.

= *Thelephora fusca* Schrader,

= *Stereum bicolor* Persoon,

= *Stereum coffeatum* Berkeley and Curtis.

Distribution and Habitat.—India — Darjeeling and Nilgiris; now collected and reported from Bengal — Calcutta, Behala, Kalighat, Bhowanipur, Alipur, Ballygunj, Tollygunj, Burdwan Districts; also Europe, United States, Canada, Cuba, New Guinea, Somerset, East Africa. Very common. Collected in July to October, 1929-32. Growing on dead trunks, logs, etc.

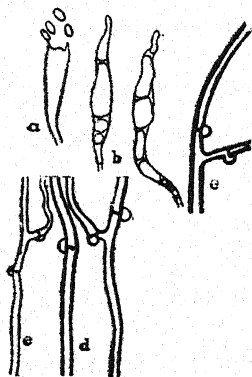


Fig. 11. a. Basidium and spores, $\times 750$; b. Gleocystidia, $\times 375$; c. Skeletal hyphæ; d. Binding hyphæ, e. Generative hyphæ, $\times 375$.

Fructification.—Generally conchate-reflexed, sometimes resupinate, often becoming imbricate, reflexed pileus about $1.5-3$ cm. long, about $1.5-5$ cm. broad and about $0.5-1$ mm. thick; dimension of resupinate specimens varies from $2-6 \times 2-3$ cm.; margin thin, entire.

Upper surface.—Villose, becoming glabrous, colour snuff-brown to bistre on drying, somewhat concentrically zoned.

Flesh.—Membranaceous, soft, spongy.

Hymenial surface.—Even, glabrous, colour at first white, ultimately becoming cream colour to pallid mouse-grey on drying, not zoned.

Spores.—Hyaline, even, dimension about $3-4 \times 2-3 \mu$.

Basidia.—Hyaline, even, clavate, dimension about $14-20 \times 5-7 \mu$.

Cystidia.—Nil.

Gleocystidia.—Present, flexuous, hyaline, or straw-coloured, colour residing in the contents, very numerous, swollen towards the base, gradually narrowed towards the apex, rising upto the hymenial layer, dimension about $20-110 \times 5-9 \mu$. (Plate IX, Fig. 50.)

Tissue differentiation.—Three distinct layers are differentiated and they are:—(a) a compact gleocystidial layer including the hymenium upto about 110μ thick, (b) an intermediate hyphal layer upto about 420μ thick, hyaline towards the hymenium and dark brown towards the hairy covering, composed of longitudinal arranged interwoven hyphæ, and (c) the outermost hairy covering, about $140-210 \mu$ thick, composed of loosely interwoven dark brown hyphæ.

Hyphal systems.—The fruit-body is of *trimitic* construction. The system of hyphæ are:—(a) Skeletal hyphæ:—Brown, thick-walled, straight or flexuous, distantly septate, very sparingly branched, longitudinal or interwoven, clamp-connection very prominent (Plate IX, Fig. 49), one almost at every septum, dimension about $4-5 \mu$ wide, wall-thickness about $1-2 \mu$, (b) Binding hyphæ:—Straw-coloured to almost hyaline, slightly thick-walled, flexuous, richly branched, septate, clamp-connections very frequent, dimension about $2.5-3.5 \mu$, wall-thickness about $.5-1 \mu$ and (c) Generative hyphæ:—Very thin-walled, flexuous, richly branched, septate, clamp-connections almost at every septum, with dense granular protoplasmic contents, dimension about $1-2 \mu$ wide.

7. *Stereum umbrinum* Berkeley and Curtis. Plate III, Fig. 24.

= *Thelephora crassa* Liv.

= *Hymenochate crassa* (Lév.) Berkeley,

= *Hymenochate umbrina* Berk. and Curtis.

= *Hymenochate vinosa* (Berk.) Cooke.

= *Hymenochate multispinulosa* Peck.

= *Hymenochate scabriseta* Cooke.

= *Lloydella scabriseta* (Cooke) v. Hohn. and Litsch.

= *Hymenochate purpurea* Cooke and Morgan.

= *Kneffia purpurea* (Cooke and Morgan) Bresadola.

= *Peniophora intermedia* Massee.

= *Hymenochate Kalchbrenneri* Massee.

Distribution and Habitat.—Now collected and reported from India, Bengal, Behala, Calcutta; also North Carolina to Texas and

Southward from Ohio and Illinois, in Arizona, West Indies and Central America; Poland, Cochin China, Australia, Africa. Rare. Collected in September 1930. Growing on fallen branches.

Fructification.—Coriaceous, somewhat spongy, generally effused, then becoming reflexed, flexible, imbricated, sometimes laterally confluent for 5 to 6 cm. reflexed portion about .5-1 cm. long, about 1-2 cm. broad and about .5 to 1 mm. thick, margin entire reflexed, thick.

Upper surface.—Velvety, somewhat concentrically zoned, covered with soft pubescence, brownish-drab to snuff-brown.

Flesh.—Coriaceous, spongy, colour same as that of the upper surface.

Hymenial surface.—Smooth, even, more or less velvety, colour snuff-brown.

Spores.—Hyaline, even, dimension about $4.6 \times 3.5-4 \mu$.

Basidia.—Hyaline, clavate, dimension about $18-22 \times 5-6 \mu$.

Cystidia.—Present, coloured, arising from the thick-walled hyphæ of the sub-hymenium, hyphæ gradually curve upwards through the hymenium as sharp pointed cystidia, wall smooth, not incrusted, dimension about $80-200 \times 4-10 \mu$.

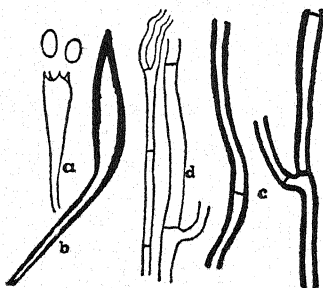


Fig. 12. *a*. Basidium and spores, $\times 750$; *b*. Cystidium, $\times 375$; *c*. Skeletal hyphæ; *d*. Thin-walled hyphæ, $\times 375$.

Tissue differentiation.—Two layers are distinguishable as follows:—(*a*) a hymenial layer containing the uppermost portion of cystidia, brown, about $56-70 \mu$ thick, (*b*) a brown hyphal layer of loosely interwoven hyphæ about $700-850 \mu$ thick. The intermediate layer and the hairy covering are undistinguishable.

Hyphal systems.—The fruit body is of *dimitic* construction. The systems of hyphæ are:—(*a*) Skeletal hyphæ:—Thick-walled, pale yellow to almost hyaline, straight or slightly flexuous, interwoven, septate, sparingly branched, about $4-8 \mu$ wide, wall-thickness about $1-2 \mu$ and (*b*) thin-walled, hyaline hyphæ, interwoven, septate, branched, without any clamp-connection, densely granulated, about $3-6 \mu$ wide.

THE COLOURED CYSTIDIA DO NOT BLACKEN ON
THE APPLICATION OF DILUTE KOH.

8. *Stereum Schomburgkii* Berkeley. Plate III, Fig. 25.

= *Lloydella Schomburgkii* (Berk.) Bres. var. *brunea* Bres.

= *Hymenochaete Schomburgkii* Massee.

Distribution and Habitat.—Now collected and reported from Bengal; also Port Darwin, Australia. Rare. Collected in September, 1930. Growing on prostrate branches.

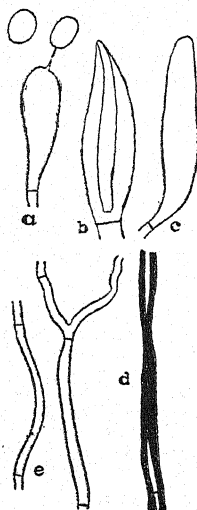


Fig. 13. a. Basidium and spores, $\times 750$; b. Cystidium, $\times 750$; c. Paraphysis, $\times 750$; d. Skeletal hyphae; e. Thin-walled hyphae, $\times 375$.

Fructification.—Thin, coriaceous, at first sub-orbicular, then reflexed, imbricate and laterally confluent, pliant, becoming somewhat stiff on drying, easily separable from the substratum, reflexed portion about 3-5 mm. long, about 7-10 mm. wide, and about .5 mm. thick, resupinate portion often extending on limbs over areas upto 20.95×16.20 mm. with reflexed areas on both sides; margin thin, entire.

Upper surface.—Velvety, covered with minute, soft pubescence, with very fine concentric zones, colour olive-brown.

Flesh.—Thin, coriaceous, olive-brown.

Hymenial surface.—Uneven, somewhat rugulose, not zoned, becoming cracked in old specimens, colour olive-brown to snuff-brown.

Spores.—Hyaline, smooth, oval, dimension about $5.6 \times 3.4 \mu$.

Basidia.—Clavate, dimension about $20 \times 6 \mu$.

Cystidia.—Present, conical, arising from the sub-hymenium, dimension about $24.40 \times 4.8 \mu$, emerging beyond the basidial layer about $10-12 \mu$.

Paraphyses.—Present, brown, cylindrical, diameter about $3-4 \mu$.

Tissue differentiation.—The tissues have been differentiated as follows:—(a) a cystidial layer including the hymenium about 40μ thick, (b) an intermediate hyphal layer, about $280-350 \mu$ thick, composed of longitudinally arranged hyphæ which bend on one side towards the hymenium, and (c) the outermost hairy covering composed of very loosely interwoven hyphæ, about $280-490 \mu$ thick.

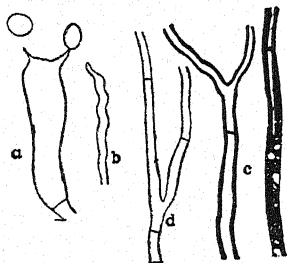


Fig. 14 a. Basidium and spores, $\times 750$; b. Paraphysis, $\times 750$; c. Skeletal hyphæ; d. Thin-walled hyphæ, $\times 375$.

Hyphal systems.—(a) Skeletal hyphæ:—Thick-walled, brown, unbranched, septate, straight or slightly flexuous, lumen almost obliterated in mature parts, about $4-5 \mu$ wide, wall-thickness about $1-2 \mu$ and (b) thin-walled, hyaline hyphæ, flexuous, interwoven, septate, sparingly branched, with richly granular protoplasmic contents, without clamp-connections, about $2-4 \mu$ wide.

9. *Stereum papyrinum* Montagne. Plate IV, Figs. 26 & 27.

= *Peniophora papyrina* (Mont.) Cooke.

= *Stereum membranaceum* Fries.

= *Stereum nicaraguense* Berk. and Curtis.

= *Stereum nicaraguæ* Berk. and Curtis.

= *Hymenochaete pallida* Cooke and Massee.

Distribution and Habitat.—India:—Ceylon, Pegu, Tineokee, Evergreen Forests; now collected and reported from Bengal, Behala, Calcutta; also Florida, West Indies, Mexico, Columbia, Brazil, Cuba, San Domingo and Africa. Common. Collected in August to September 1930. Growing on dead branches of trees.

Fructification.—Thin, papery, spongy, coriaceous, flexible, resupinate, generally broadly effused, then reflexed, sometimes imbricate, laterally confluent, resupinate patches at first more or less orbicular,

then becoming confluent, reflexed portion about 4-12 mm. long, about 10-22 mm. broad and about .5 mm. thick, resupinate portion at first about 10-25 mm. across, sometimes on over areas upto $260 \times 35-55$ mm. with reflexed areas on both sides; margin entire, involuted when dry.

Upper surface.—Velvety, densely tomentose, very faintly zoned, colour snuff-brown to cartridge-buff.

Flesh.—Thin, coriaceous.

Hymenial surface.—Smooth, velvety, faintly zonate, colour snuff-brown to benzo brown, often with a violaceous tint.

Spores.—Hyaline, smooth, sub-globose, dimension about $4-5 \times 3 \mu$.

Basidia.—Simple, hyaline, cylindrical, dimension about $26-28 \times 6-8 \mu$.

Cystidia.—Present, conical, heavily and coarsely incrustated all over, usually coloured pale yellow under the incrustations, very numerous, arising both from the hymenial and sub-hymenial layers, dimension about $50-90 \times 13-20 \mu$ emerging upto 31μ (Plate IX, Fig. 52).

Paraphyses.—Present, flexuous, diameter about 2μ .

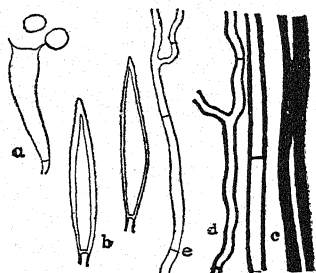


Fig. 15. *a*. Basidium and spores; *b*. Cystidia; *c*. Skeletal hyphae, *d*. Binding hyphae; *e*. Thin-walled hyphae; $\times 375$.

Tissue differentiation.—Three layers are distinctly differentiated as follows:—(*a*) a compact cystidial layer along with the hymenial layer, about 90μ thick, (*b*) an intermediate hyphal layer of longitudinally arranged densely inter-woven hyphae, hyaline towards the hymenium, about 140μ thick, and (*c*) the outermost hairy covering, composed of loosely interwoven hyphae, about 280μ thick.

Hyphal systems.—Fruit-body is of *dimitic* construction. The systems of hyphae are:—(*a*) Skeletal hyphae:—Thick-walled, brown, straight or flexuous, sparingly branched, longitudinal, distantly septate, lumen almost obliterated in mature parts, about $4-6 \mu$ wide,

wall-thickness about $1.2.5 \mu$ and (b) thin-walled, hyaline hyphæ, closely septate, sparingly branched, interwoven, with dense protoplasmic contents, without clamp-connection, about 4.5μ wide.

10. *Stereum endocrocinum* Berkeley. Plate IV, Fig. 28.

Distribution and Habitat.—India:—Yaugma Valley, E. Nepal, now collected and reported from Bengal — Behala. Rare. Collected in September 1931. Growing on dead branches of trees.

Fructification.—Thin, coriaceous, papery, effuso-reflexed, sometimes imbricated and laterally confluent, pliant, reflexed portion about 10-14 mm. long, about 10-25 mm. broad and about $.5 \text{ mm.}$ thick, resupinate portion often extending over areas upto $180 \times 20.25 \text{ mm.}$ with reflexed areas along both sides; margin entire, thin.

Upper surface.—Velvety, densely covered with soft pubescence, faintly concentrically sulcate, colour snuff-brown to pale brownish grey on drying.

Flesh.—Thin, coriaceous.

Hymenial surface.—Uneven, finely radially ridged near the margin of the reflexed pileus, not zoned, somewhat rugulose, colour cinnamon to rufous brown, velvety or ochraceous.

Spores.—Hyaline, smooth, sub-globose, dimension about 4μ .

Basidia.—Hyaline, clavate, dimension about $18.24 \times 5.8 \mu$.

Cystidia.—Present, hyaline, not incrustated, more or less conical, thick-walled, apex blunt to sharply pointed, arising from the hymenium dimension about $30.50 \times 10.12 \mu$, emerging upto 30μ .

Tissue differentiation.—Three distinct layers are differentiated as follows.—(a) the hymenial layer along with cystidia, about 30.50μ thick, (b) an intermediate hyphal layer, upto about 280μ thick, hyaline towards the hymenium and dark-brown towards the hymenium, composed of longitudinally arranged interwoven, hyphæ, and (c) the outermost hairy covering composed of loosely interwoven hyphæ, about 140.420μ thick.

Hyphal systems.—Fruit-body is of *trimitic* construction. The systems of hyphæ are:—(a) Skeletal hyphæ:—Very thick-walled, brown hyphæ, straight or slightly flexuous, distantly septate, longitudinal, lumen more or less obliterated in mature parts, about 4.8μ wide, wall-thickness about $1.3.5 \mu$, (b) Binding hyphæ:—Thick-walled, hyaline, flexuous, septate, sparingly branched, interwoven, about 3.4μ wide, wall-thickness about 1μ and (c) Thin-walled, hyaline hyphæ, septate branched, with dense protoplasmic contents, about 2.4μ wide, without any clamp-connection.

11. *Stereum scytale* Berkeley. Plate V, Figs. 29 & 30.

Distribution and Habitat.—India — Khasia Mts. (Drs. Hooker and Thomson), West Himalayas (General Strachey), Sikkim, now collected and reported from Bengal-Calcutta, Behala, Burdwan and Darjeeling Districts; also Pegu, Yomah (Kurz), Brazil, Japan and

Cuba. Common. Collected in September to November 1930-31. Growing on dead logs.

Fructification.—Of rigid coriaceous substance, but rather flexible, becoming hard and brittle when dry, easily becoming cracked from base to the margin, sessile, dimidiate, applanate, sometimes imbricated and laterally confluent, about 3.5-7.18 cm. long about 2.5-8-20 cm. broad, and about 1-2 mm. thick; margin entire.

Upper surface.—Finely velvety or pubescent, older portions becoming rough due to the hairs falling off, with prominent narrow, raised, concentric zones, marked with longitudinal wrinkles, especially prominent in larger and thicker specimens, colour deep brown to reddish brown.

Flesh.—Rigid coriaceous, brown.

Hymenial surface.—Uneven, with raised pustules sometimes zonate, finely wrinkled near the edge, colour range includes ochraceous, umber.

Spores.—Hyaline, smooth, sub-globose, dimension about $5-6 \times 5 \mu$.

Paraphyses.—Present, conspicuous, "bottle-brush" type, clavate or cylindrical, very numerous forming a compact layer covering the basidial layer, dimension about $20-30 \times 3-6 \mu$ (Plate IX, Fig. 47).

Cystidia.—Nil.

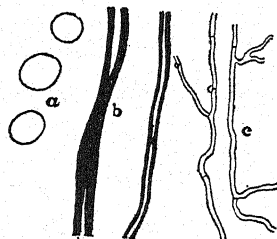


Fig. 16. *a*. Spores, $\times 750$; *b*. Skeletal hyphae; *c*. Generative hyphae, $\times 375$.

Tissue differentiation.—The following layers of tissues are recognisable:—(a) a more or less hyaline hymenial layer containing numerous "bottle-brush" paraphyses about 30μ thick, followed by, (b) several alternating closely laminated pale yellow and dark brown layers, varying in number and also in thickness, total thickness about $140-210 \mu$ thick, composed of sub-erect interwoven hyphae, (c) a broad intermediate hyphal layer, about $560-630 \mu$ thick, composed of longitudinally arranged subparallel hyphae, (d) a dark brown compact hyphal layer longitudinally arranged, about $100-140 \mu$ thick, forming the crust subtending the hairy covering, and (e) the outermost hairy covering about $140-360 \mu$ thick, composed of interwoven erect hyphae.

Hyphal systems.—The fruit-body is of *dimitic* construction. The systems of hyphæ are as follows:—(a) Skeletal hyphæ:—Thick-walled, brown to almost septate, unbranched, longitudinal, about 3-6 μ wide, wall-thickness about 1-2 μ , lumen almost obliterated in mature parts, (b) Binding hyphæ:—Not differentiated, (c) Generative hyphæ:—Thin-walled, hyaline, flexuous, interwoven, richly branched, closely septate, with a few clamp-connections, about 1-2 μ wide, with dense granular protoplasmic contents.

12. *Stereum percome* Berkeley and Broome.

Plate V, Figs. 31 & 32.

= *Lloydella papyracea* (Jungh.) Bres.

Distribution and Habitat.—India:—Now collected and reported from Bengal-Calcutta, Behala, Alipur, Kalighat, Belgachia, Ballygunj, Burdwan and Darjeeling districts; also Ceylon, Central Provinces and Cape. Very common. Collected in July to October 1929-32. Growing on dead branches of trees and logs.

Fructification.—Sessile, effuso-reflexed, at first orbicular and resupinate with a delicate white margin, often becoming densely imbricated, laterally confluent, resupinate patches soon become confluent forming a broad continuous sheet, reflexed portion often become much folded; the reflexed pileus about 1.5 to 5 cm. long, about 3 to 10 cm. broad and about 1.5 to 2 mm. thick, dimension of the resupinate patches often attaining several feet, leathery, tough; margin wavy, colour variable, such as white to lavender.

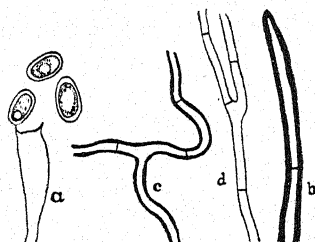


Fig. 17. a. Basidium and spores, $\times 750$; b. Skeletal hyphæ; c. Binding hyphæ; d. Thin-walled hyphæ, $\times 375$.

Upper surface.—Rough, covered with strongly strigose hairs, concentrically sulcate, colour pale brownish-grey.

Flesh.—Leathery about 1 mm. thick, colour same as that of the hymenium.

Hymenial surface.—Uneven, with raised ridges and folds, concentrically zoned, granular, the range of colour includes yellow-brown, cinnamon, rufous brown and fawn brown; sometimes with a narrow purple brown zone just behind the edge.

Spores.—Hyaline, oval, smooth, dimension about $7.8 \times 4.6 \mu$.

Basidia.—Hyaline, clavate, dimension about $20.26 \times 5.6 \mu$.

Cystidia.—Present, numerous, conical, hyaline, heavily half-incrusted, arising mostly from the sub-hymenium, dimension about $40.70 \times 16.24 \mu$, emerging about 10.30μ , (Plate IX, Fig. 51).

Tissue differentiation.—The tissues have been differentiated as follows:—(a) a cystidal layer including the hymenium upto 70μ thick, (b) an intermediate hyphal layer which bend on one side towards the hymenium, dark brown towards the upper surface, composed of longitudinally arranged interwoven hyphæ, about 420.560μ thick, and (c) the outermost hairy covering, about 280.1260μ thick, composed of loosely interlaced hyphæ.

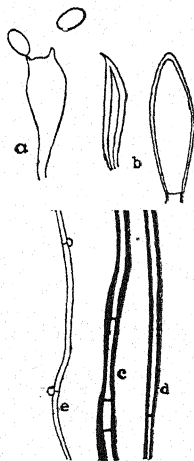


Fig. 18. a. Basidium and spores, $\times 750$; b. Cystidia, $\times 750$; c. Skeletal hyphæ, $\times 375$; d. Binding hyphæ, $\times 375$; e. Generative hyphæ, $\times 750$.

Hyphal system.—The fruit body is of *trimitic* construction. The systems of hyphæ are as follows:—(a) Skeletal hyphæ:—Dark-brown, thick-walled, straight, unbranched, septate, about 5.6μ wide, wall-thickness about $2.2.5 \mu$, (b) Binding hyphæ:—Thick-walled hyaline, flexuous, interwoven, septate, richly branched about 4.5μ wide, wall-thickness about $1.1.5 \mu$ and (c) thin-walled, hyaline hyphæ, with densely granular protoplasmic contents, branched, septate, interwoven, without any clamp-connection, about 3.4μ wide.

13. *Stereum vibrans* Berkeley and Curtis. Plate VI, Figs. 33 & 34.

Distribution and Habitat.—Now collected and reported from India—Bengal-Calcutta; also Cuba, Jamaica. Rare. Collected in October, 1930. Growing on logs.

Fructification.—Thin, of rigid coriaceous substance, dimidiate, flabelliform, concentrically sulcate, attached to the host by the narrowed base, imbricate, flexible, often laterally confluent, lobed, about 5-6 cm. long, about 7-8 cm. broad and about .5 to 1 mm. thick; margin entire.

Upper surface.—Velvety, covered with minute dense pubescence, persisting near the margin, older portions in old specimens becoming glabrous exposing the blackish horny crust near the region of attachment; with distinct concentric zones, colour varying from snuff-brown to Saccardo's umber.

Flesh.—Rigid, coriaceous, colours Saccardo's umber.

Hymenial surface.—Even, smooth, faintly zoned, sometimes faintly wrinkled especially near the margin, colour Saccardo's umber to drab.

Spores.—Hyaline, oval, smooth, dimension about $5-6 \times 3 \mu$.

Basidia.—Simple, clavate, hyaline, dimension about $18-20 \times 6 \mu$.

Cystidia.—Present, very few, hyaline, conical, with a pointed apex, apex sometimes curved, arising from the hymenium, dimension about $20-26 \times 6-10 \mu$.

Tissue differentiation.—Four layers are differentiated as follows:—(a) a hyaline hymenial layer, about 20μ thick, (b) a compact intermediate hyphal layer, about 350μ thick, pale yellow-brown, composed of longitudinally arranged sub-parallel hyphæ, (c) a dark brown layer just behind the hairy covering, about $84-210 \mu$ thick, composed of matted longitudinal hyphæ, and bearing, (d) the outermost hairy covering, about 280μ thick composed of loosely interwoven hyphæ.

Hyphal systems.—The fruit-body is of *trimitic* construction. The different systems of hyphæ are:—(a) Skeletal hyphæ:—Very thick-walled, yellow-brown, straight or curved, longitudinal, unbranched, closely septate, lumen almost obliterated in mature parts, about $5-8 \mu$ wide, wall-thickness about $2-3 \mu$, (b) Binding hyphæ:—Thick-walled hyaline, flexuous interwoven or straight, distantly septate, unbranched about $2-4 \mu$ wide, wall-thickness about 1.5μ and (c) Generative hyphæ:—Thin-walled, hyaline, flexuous, septate, sparingly branched, with distinct clamp-connections, densely granular protoplasm, interwoven, about $1-2 \mu$ wide.

SECTIONS BLACKEN BY THE ACTION OF DILUTE KOH.

14. *Stereum alternum* Lloyd. Plate VI, Figs. 35 & 36.

Distribution and Habitat.—India — Now collected and reported from Bengal, Darjeeling. Rare. Collected in September. 1931. Growing on dead wood.

Fructification.—Of rigid coriaceous substance, becoming hard and brittle when dry, easily becoming cracked, sessile, dimidiate applanate, attached to the substratum by a narrow point, about 5-7 mm. long, about 10-15 mm. broad, and about 1-2 mm. thick; margin entire.

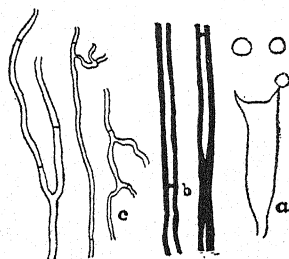


Fig. 19. *a.* Basidium and spores, $\times 750$; *b.* Skeletal hyphae, $\times 375$; *c.* Thin-walled hyphae, $\times 375$.

Upper surface.—Finely velvety or pubescent, older portions becoming rough due to the hairs falling off, concentrically sulcate, zones very narrow and prominent near the margin, colour fuscus.

Flesh.—Thick, rigid, coriaceous, brown.

Hymenial surface.—Even, not zoned, colour whitish, sometimes hazel.

Spores.—Hyaline, smooth, sub-globose, dimension about $3-4 \times 3 \mu$.

Basidia.—Simple, hyaline, cylindrical, dimension about $18-20 \times 6-8 \mu$.

Cystidia.—Nil.

Paraphyses.—Very conspicuous, “bottle-brush” type, clavate or cylindrical, hyaline, dimension about $14-22 \times 4-10 \mu$.

Tissue differentiation.—The tissues are differentiated into several closely laminated areas, consisting of pallid, pale yellow or dark-brown, sub-translucent layers alternating with the hyaline layers. The hyaline layers are not regular but broaden out here and there (about $30-420 \mu$ wide). The hyphae are densely interwoven and gradually bend towards the upper surface forming an erect or sub-erect dark-brown compact hyphal layer.

Hyphal systems.—(*a*) Skeletal hyphae:—Thick-walled, yellow-brown, straight or slightly flexuous, distantly septate, unbranched, lumen almost obliterated in mature parts, about $3-6 \mu$ wide, wall-thickness about $1-2 \mu$ and (*b*) thin-walled, hyaline hyphae, septate,

richly branched, interwoven, with dense granular protoplasm, without any clamp-connection, about 1-3 μ wide.

Lloyd remarks that this species is the pileate form of *Stereum annosum*.

15. *Stereum annosum* Berkeley and Broome.

Plate VI, Figs. 37 & 38.

Distribution and Habitat.—India, Ceylon; now collected and reported from Bengal, Behala and Hooghly districts. Rare. Collected in September 1930. Growing on dead branches of trees.

Fructification.—Of rigid coriaceous substance, becoming hard and brittle when dry, easily becoming cracked, generally in the form of oval or irregular thick plates, effuso-reflexed undulating and partly free, resupinate part sometimes extending in a continuous layer for about 8 cm. along a branch, reflexed pilei upto about 3.5 cm. long, about 5.5 cm. broad and about 3-4 mm. thick; margin thick, entire, rounded.

Upper surface.—Velvety, older portions becoming rough due to the hairs falling off, concentrically sulcate, ochraceous, or dark-brown to blackish when dry.

Flesh.—Stiff, coriaceous, closely laminated, brown, about 2-4 mm. thick.

Hymenial surface.—Uneven, with the depressed zones of the pileus showing on its upper surface, colour pale pinkish buff.

Anatomical characters same as *S. alternum*.

16. *Stereum fasciatum* Schweinitz. Plate VII, Figs. 39 & 40.

= *Thelephora vericolor* B. *fasciata* (Schw.) Fr.

= *Thelephora ostrea* Blume and Nees.

= *Stereum Ostrea* (Blume and Nees) Fries.

= *Thelephora* (*Stereum*) *Mollis* Lévillé.

= *Stereum Molle* Lévillé.

= *Stereum arcticum* Fries.

Distribution and Habitat.—India:—Nilgiris, Sikkim Himalayas; now collected and reported from Bengal, Calcutta and Darjeeling Districts; also United States, Cuba, Jamaica, Mexico, San Domingo, Canada, West Indies, South America, Norway, Sweden, Formosa, Java, Japan, New Zealand, Madeira, Madagascar. Rare. Collected in September, 1930. Growing on logs.

Fructification.—Thin, or rigid coriaceous substance, umbonato-sessile, broadly effused, then reflexed, sometimes laterally confluent, flexible, becoming cracked near the margin when dry; reflexed portion flabelliform, dimidiate, sometimes tapering towards the base,

about 3.5-8 cm. long, about 3.5-12 cm. broad and about .5 mm. thick; margin entire.

Upper surface.—Velvety, marked with fine concentric zones, densely tomentose, colour on drying warm-buff, tawny olive and snuff-brown, in weathered specimens with tomentum torn apart in narrow zones exposing the stiff, bared, chestnut surface, sometimes with a violet-black tinge; very often the snuff-brown or bay-brown zones alternating with warm-buff zones.

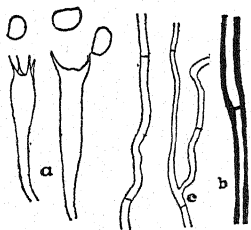


Fig. 20. *a.* Basidia and spores, $\times 750$; *b.* Skeletal hyphae, $\times 375$; *c.* Thin-walled hyphae; $\times 375$.

Flesh.—Thin, coriaceous, warm-buff.

Hymenial surface.—Smooth, faintly zoned, sometimes with faint radial ridges, colour usually warm-buff, cinnamon, sometimes with reddish, lilac or violaceous tints, especially near the margin.

Spores.—Hyaline, flattened on one side, smooth, dimension about $4.6 \times 3.3.5 \mu$ as seen on basidia.

Basidia.—Hyaline, cylindrical or clavate; dimension about $20-24 \times 5.6 \mu$.

Tissue differentiation.—Four layers are differentiated as follows:—(a) a hyaline hymenial layer, about 24μ thick, (b) an intermediate hyphal layer, about $280-490 \mu$ thick, composed of very densely arranged longitudinal hyphae; pale yellow and straw-coloured, which is bordered on the outer side towards the hairy covering by, (c) a darker yellow-brown layer about $42-50 \mu$ thick, composed of densely matted longitudinal hyphae, and (d) the outermost hairy covering, about 280μ thick, composed of very loosely interwoven hyphae, pale yellow.

Hyphal systems.—The fruit-body is of *dimitic* construction. The hyphal systems are:—(a) Skeletal hyphae:—Thick-wall, straight or slightly flexuous, pale yellow, septate, about $4-6 \mu$ wide, wall-thickness about $1-2 \mu$ and (b) thin-walled, hyaline hyphae, closely septate, interwoven, sparingly branched, without clamp-connections, with dense protoplasmic contents, about $1-2 \mu$ wide.

SECTIONS OF THE FRUIT-BODIES DARKEN ON THE
APPLICATION OF DILUTE KOH.

17. *Stereum hirsutum* Willdenow ex Fries. Plate VIII, Figs. 41-44.

- = *Thelephora hirsuta* Willd.
- = *Auricularia reflexa* Bull.
- = *Thelephora ochracea* Schweinitz.
- = *Thelephora subzonata* Fries.
- = *Corticium subzonatum* Fries.
- = *Stereum variicolor* Lloyd.

Distribution and Habitat.—India:—Darjeeling and Lebong (Hooker f.), Arnigadh, Mussoorie (Gollan); Khandala, Bombay (Blatter); N. W. Himalayas (W. T. Saxton); India (Cave); Sonamarg, Kashmir (R. R. Stewart); now collected and reported from Bengal-Calcutta, Behala; also Europe, Britain, France, United States, British N. America, Vancouver Islands, Mexico; Ecuador, Cuba, Venezuela, Australia, Tasmania, New Zealand, Chatham Islands, Chili, Java, Africa. Common. Collected in July to October 1930-31. Growing on decaying trunks, branches, stumps, logs, etc.

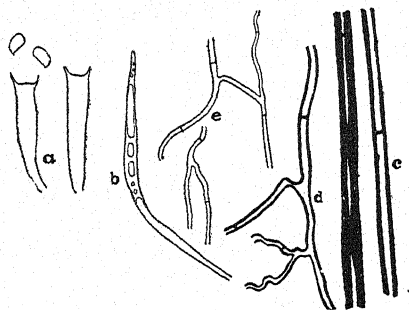


Fig. 21. a. Basidia and spores, $\times 750$; b. Lactiferous cell, $\times 375$; c. Skeletal hyphae; d. Binding hyphae; e. Generative hyphae, $\times 375$.

Fructification.—Sessile, of stiff coriaceous substance, form very variable, very broadly effused, then reflexed, very rarely wholly resupinate, becoming much folded when dry, flexible, densely imbricate, very often laterally confluent, reflexed portion about 1.5 to 4.5 cm. long, about 3 to 8 cm. broad, and about .5 to 2 mm. thick; margin entire, involuted when dry, colour yellowish.

Upper surface.—Covered with dense strigose hairs, somewhat concentrically sulcate, colour varying shades of grey, cream-buff or pallid, old and weathered specimens becoming greyish with a thin, hardened crust-like surface.

Flesh.—Coriaceous, stiff, yellowish.

Hymenial surface.—Even, smooth, sometimes very faintly zoned, folded when dry, colour warm-buff, smoke grey, pinkish or tan colour.

Spores.—White, smooth, flattened on one side, apiculus present, dimension about $4.6 \times 2.5 \mu$.

Basidia.—Hyaline, cylindrical, dimensions about $18-20 \times 4.6 \mu$.

Cystidia.—Nil.

Gleocystidia.—Nil.

Lactiferous cells.—Present, frequent, contents coloured, elongated, arising from the sub-hymenium dimension about $60-120 \times 4.6 \mu$.

Tissue differentiation.—Four distinct layers are recognised as follows:—(a) a layer composed of conducting organs along with the hymenium, $170-120 \mu$ thick, (b) an intermediate layer of longitudinally arranged sub-parallel hyphæ, which bend on one side into the hymenium, upto about 280μ thick, (c) a golden yellow zone just below the hairy covering, composed of longitudinally arranged hyphæ, about $28-70 \mu$ thick, and (d) the outermost hairy covering, composed of densely interwoven hyphæ, about $168-210 \mu$ thick.

Hyphal systems.—Fruit-body is of *trimitic* construction. The systems of hyphæ are:—(a) Skeletal hyphæ:—Thick-walled, pale yellow-brown hyphæ, longitudinal, straight, or slightly flexuous, unbranched, distantly septate, lumen almost obliterated in mature parts, about $4-6 \mu$ wide, wall-thickness about $1-3 \mu$, (b) Binding hyphæ:—Thick-walled, almost hyaline, flexuous, branched, interwoven, distantly septate, coarsely granular, about $2-4 \mu$ wide, wall-thickness about $5-1 \mu$ and (c) Generative hyphæ:—Thin-walled, hyaline, richly branched, septate, interwoven, densely granular, about $1-2 \mu$ wide, H-connection present, few.

Asterostromella von Höhnelt and Litschauer. Plate VIII, Fig. 45.

Fructifications broad, easily cracking, scaly, thin, membranaceous, waxy, closely adpressed to the substratum. Hymenium uneven, consisting of basidia and paraphyses; paraphyses forming a sort of a felt and dichotomously branched (dichophyses), colourless or slightly coloured, terminal branches being very pointed. Basidia cylindrical with 2-4 sterigmata. Spore hyaline, smooth, thin-walled.

Asterostromella rhodospora Wakefield. Plate VIII, Fig. 45.

Distribution and Habitat.—Now collected and reported from India, Bengal, Ballygunj and Tollygunj; also Australia and New Zealand. Common. Collected in August to September, 1930-32. Growing on dead branches of trees.

Fructification.—Thin, coriaceous, rigid, becoming hard and brittle when dry, easily cracking, very broadly effused, closely adpressed to the substratum, running over the rough wood and surrounding every projecting point, extending over areas upto a foot, margin thin whitish. Flesh white, coriaceous, rigid. Hymenium uneven, rugulose, dry, colour dirty white.

Spores.—Hyaline, globose, smooth, dimension about $1.2\ \mu$.

Basidia.—Clavate, hyaline dimension about $6.8 \times 2.5\ \mu$.

Paraphyses.—Present, much branched in a dichotomous manner.

Tissue differentiation.—The subiculum is differentiated into several alternating hyaline and brownish layers, varying in thickness, total thickness about $700\text{--}840\ \mu$.

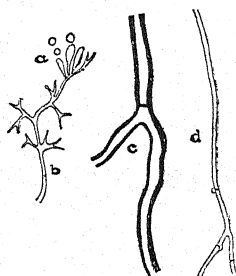


Fig. 22. a. Basidia and spores; b. Branched paraphysis; c. Skeletal hyphæ; d. Generative hyphæ; $\times 375$.

Hyphal systems.—The fruit-body is *dimitic* in construction. The systems of hyphæ are:—(a) Skeletal hyphæ:—Thick-walled, hyaline, septate, sparingly branched, flexuous interwoven, about $4\text{--}6\ \mu$ wide, wall-thickness about $1\text{--}1.5\ \mu$, and (b) Generative hyphæ:—Thin walled, hyaline, much branched, septate, with clamp-connections, densely granular protoplasmic contents, interwoven, about $1\text{--}2\ \mu$ wide.

Craterellus Persoon.

Fructifications fleshy or membranaceous, pileate, stipitate, tubiform, infundibuliform or flabelliform, or unbilicate. Stem central, confluent with the pileus. Hymenium even, rugose, becoming wrinkled, waxy-membranous. Basidia simple, with 2-4 sterigmata. Spores generally white. Growing on ground.

Craterellus cornucopioides (Linn.) Persoon.

= *Peziza cornucopioides* L.

= *Elvella cornucopioides* Scop.

= *Merulius cornucopioides* Pers.

= *Cantharellus cornucopioides* Fries.

Distribution and Habitat.—Now collected and reported from India, Bengal, Hooghly district; also Canada to South Carolina and Westward to Missouri. Rare. Growing on ground.

Fructification.—Gregarious, thin, membranaceous, tubaeform, pervious, about 5 cm. high, about 3 cm. broad, margin decurrent, generally lobed.

Upper surface.—Squamulose, smoky-brown or blackish, drying Prout's brown.

Hymenial surface.—Rugose-wrinkled, cineaceous drab.

Stem.—Short, hollow, blackish, dimension about 1-2 cm.

Spores.—Hyaline, even, oblong, elliptical, dimension about $10-16 \times 6-8 \mu$.

It is an edible species and suggests an affinity with *Cantharellus* of the *Agaricaceæ*.

EXPLANATION OF PLATES

PLATE I.

- Fig. 1.—*Hymenochæte aspera*. Upper surface.
Fig. 2.—*Hymenochæte aspera*. Hymenial surface.
Fig. 3.—*Hymenochæte tenuissima*. Upper surface.
Fig. 4.—*Hymenochæte tenuissima*. Hymenial surface.
Fig. 5.—*Hymenochæte rubiginosa*. Upper surface.
Fig. 6.—*Hymenochæte rubiginosa*. Hymenial surface.
Fig. 7.—*Hymenochæte nigricans*. Upper surface.
Fig. 8.—*Hymenochæte nigricans*. Hymenial surface.
Figs. 9 & 10.—*Hymenochæte cacao*. Fructifications.

PLATE II.

- Fig. 11.—*Stereum elegans*.
Fig. 12.—*Stereum nitidulum*.
Fig. 13.—*Stereum petalodes*. Upper surface.
Fig. 14.—*Stereum petalodes*. Hymenial surface.
Fig. 15.—*Stereum crenatum*.
Fig. 16.—*Stereum glabrescens*. Upper surface.
Fig. 17.—*Stereum glabrescens*. Hymenial surface.

PLATE III.

- Figs. 18-20.—*Stereum fuscum*. Upper surface.
Figs. 21-23.—*Stereum fuscum*. Hymenial surface.
Fig. 24.—*Stereum umbrinum*.
Fig. 25.—*Stereum Schomburgkii*.

PLATE IV.

- Fig. 26.—*Stereum papyrinum*. Fructification effuso-reflexed.
Fig. 27.—*Stereum papyrinum*. Fructification resupinate.
Fig. 28.—*Stereum endocrocinum*. Hymenial surface.

PLATE V.

- Fig. 29.—*Stereum scytale*. Upper surface.
 Fig. 30.—*Stereum scytale*. Hymenial surface.
 Fig. 31.—*Stereum percome*. Upper surface.
 Fig. 32.—*Stereum percome*. Hymenial surface.

PLATE VI.

- Fig. 33.—*Stereum vibrans*. Upper surface.
 Fig. 34.—*Stereum vibrans*. Hymenial surface.
 Fig. 35.—*Stereum alternum*. Upper surface.
 Fig. 36.—*Stereum alternum*. Hymenial surface.
 Fig. 37.—*Stereum annosum*. Upper surface.
 Fig. 38.—*Stereum annosum*. Hymenial surface.

PLATE VII.

- Fig. 39.—*Stereum fasciatum*. Upper surface.
 Fig. 40.—*Stereum fasciatum*. Hymenial surface.

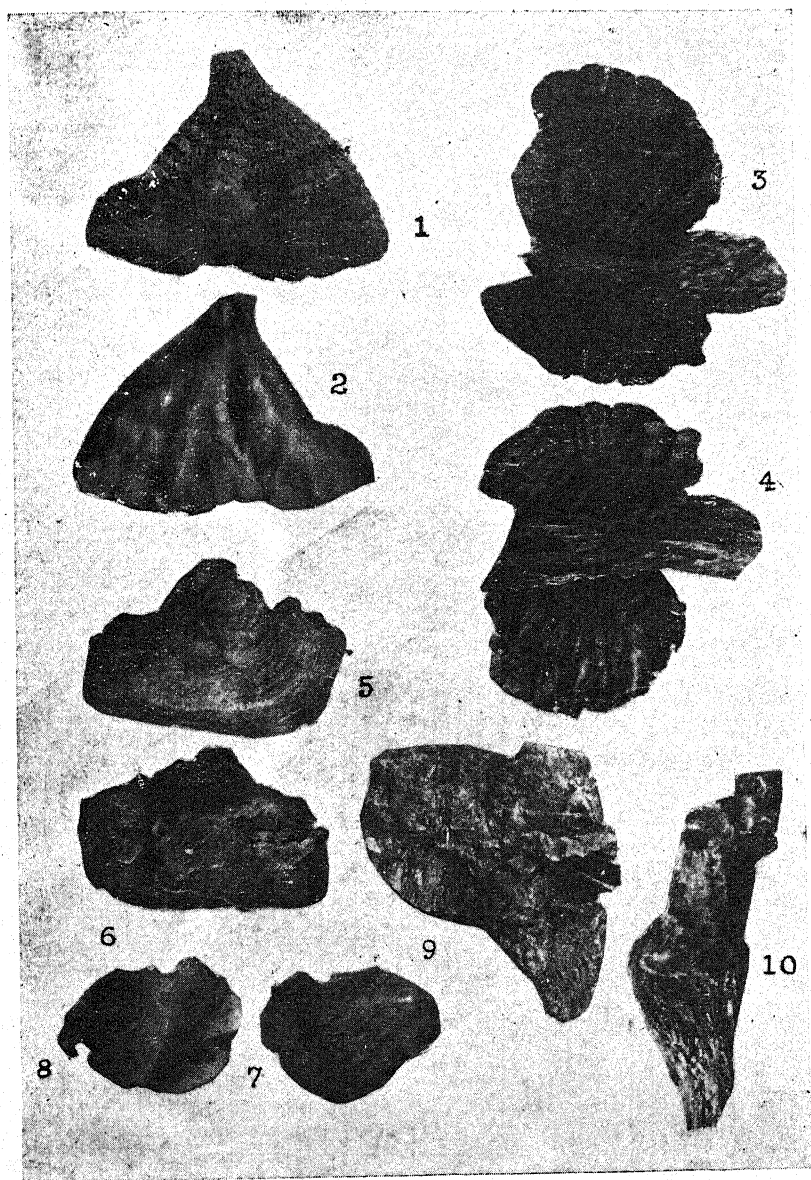
PLATE VIII.

- Figs. 41 & 42.—*Stereum hirsutum*. Upper surface.
 Fig. 43.—*Stereum hirsutum*. Hymenial surface.
 Fig. 44.—*Stereum hirsutum*. Fructification effuso-reflexed.
 Fig. 45.—*Asterostromella rhodospora*.

PLATE IX.

- Fig. 46.—*Hymenochæte Cacao*. Photomicrograph showing the setigerous layer; $\times 510$.
 Fig. 47.—*Stereum scytale*. Photomicrograph showing "bottle-brush" paraphyses; $\times 1020$.
 Fig. 48.—*Hymenochæte tenuissima*. Photomicrograph showing the setigerous layer; $\times 510$.
 Fig. 49.—*Stereum fuscum*. Photomicrograph showing a clamped hypha; $\times 1020$.
 Fig. 50.—*Stereum fuscum*. Photomicrograph showing gleocystidia; (a) gleocystidia; $\times 510$.
 Fig. 51.—*Stereum percome*. Photomicrograph showing incrustated cystidia; $\times 510$.
 Fig. 52.—*Stereum papyrinum*. Photomicrograph showing the cystidial layer; cystidia incrustated; $\times 510$.
 Fig. 53.—*Hymenochæte nigricans*. Photomicrograph showing the setigerous layer; $\times 510$.
 Fig. 54. *Stereum nitidulum*. Photomicrograph showing the hymenial layer; (a) gleocystidia; $\times 250$.

Plate I



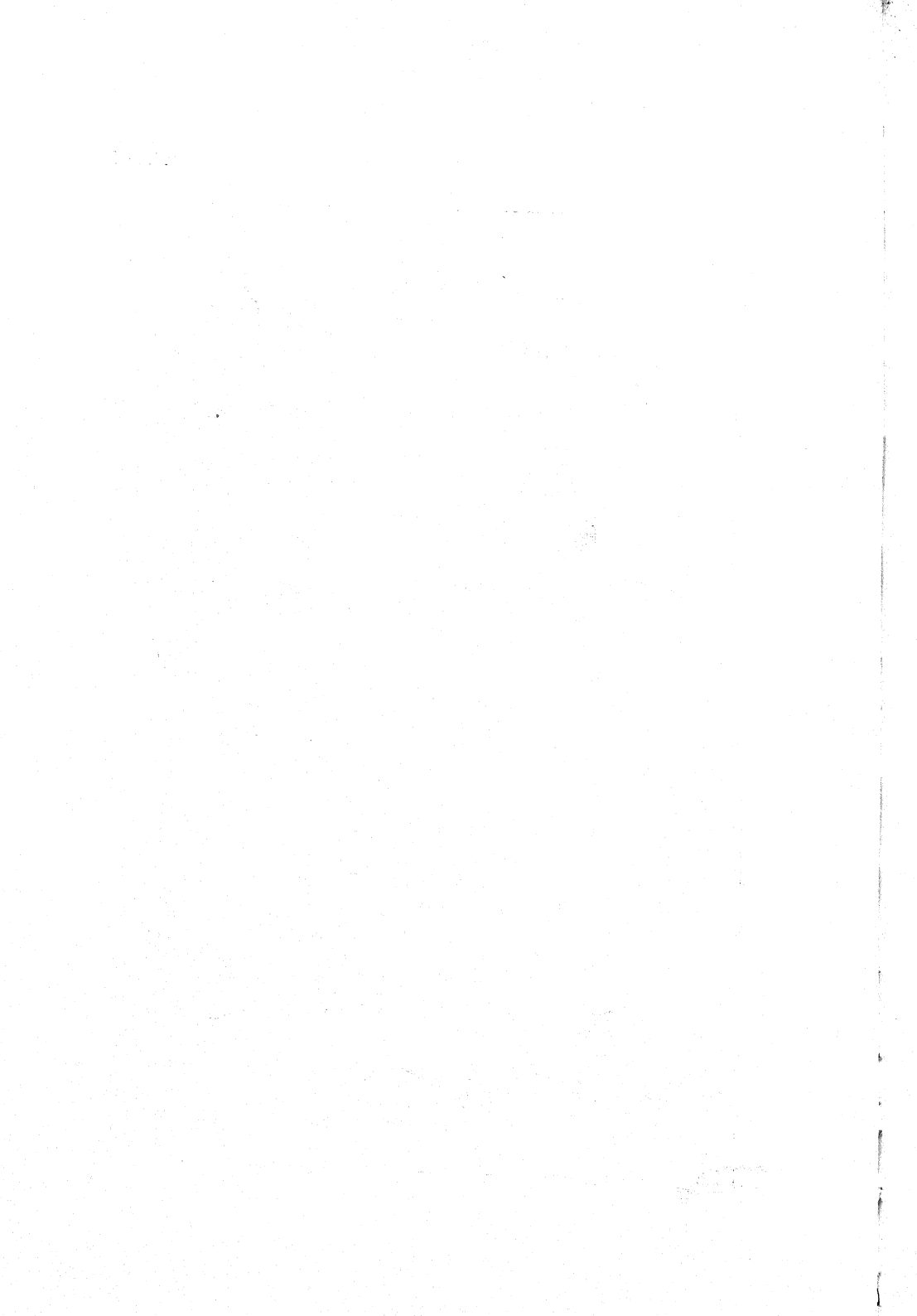
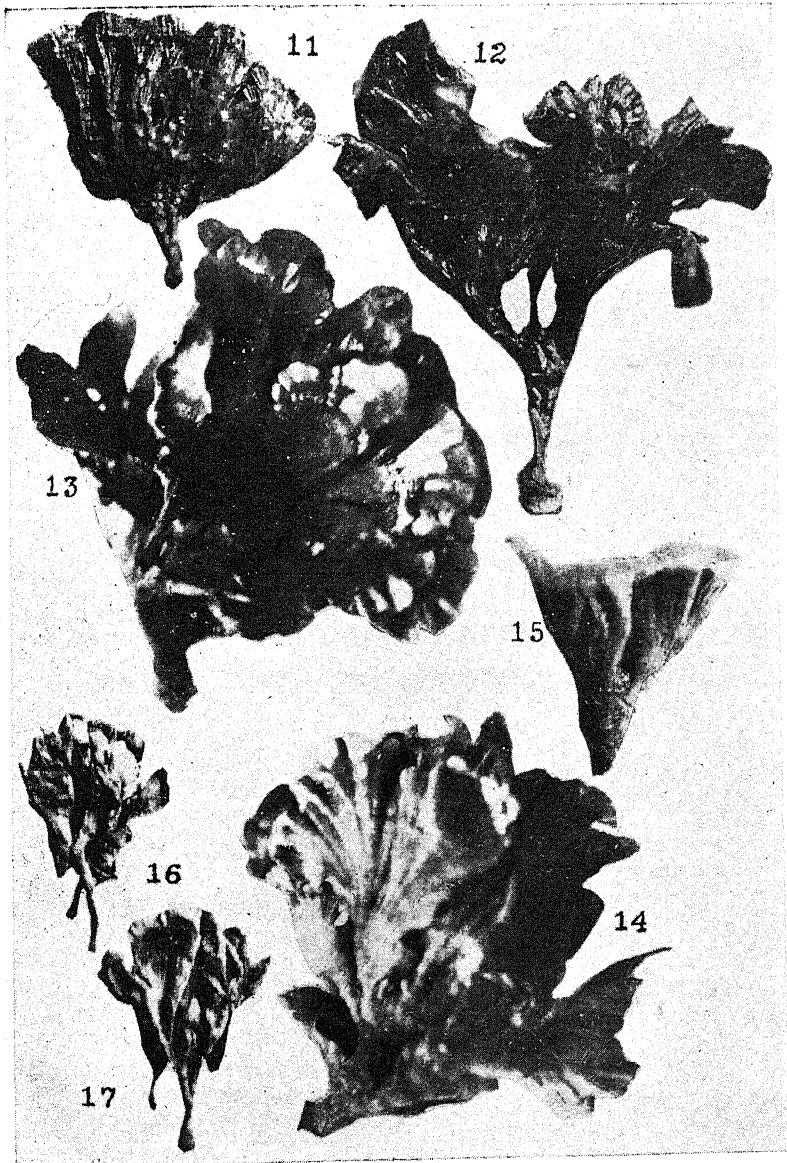
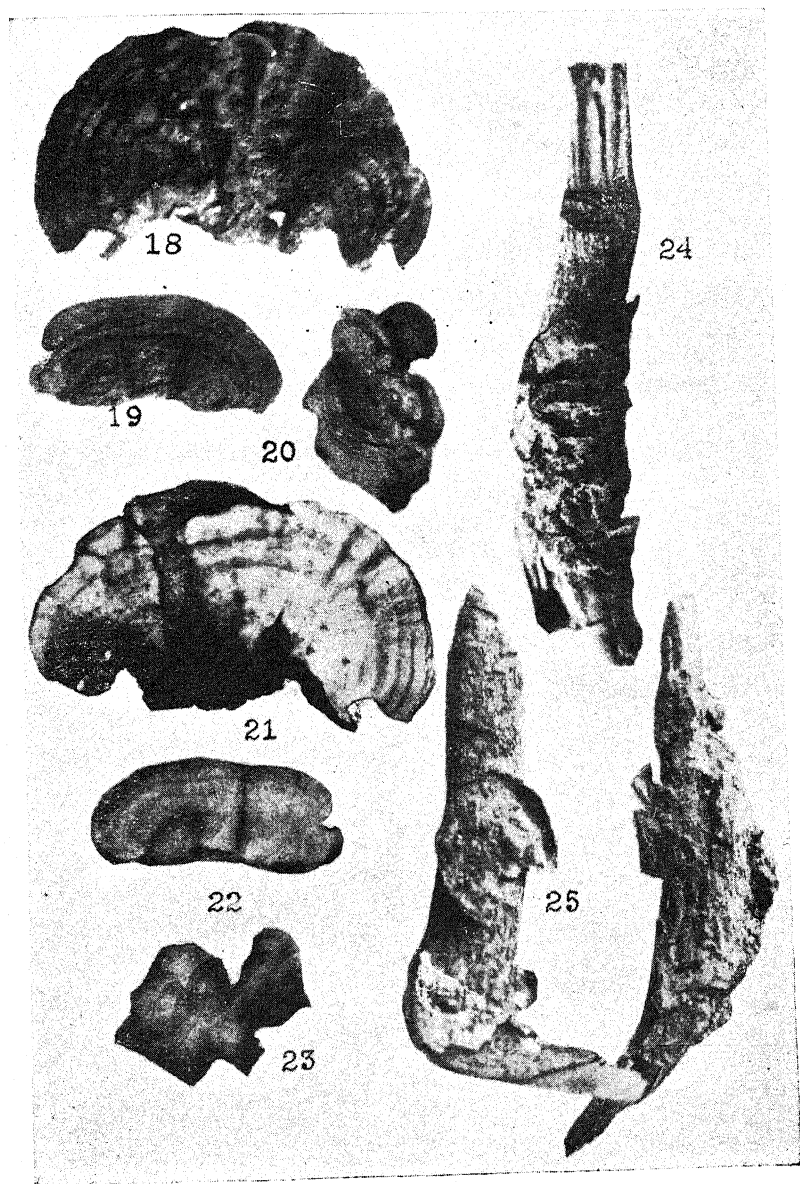
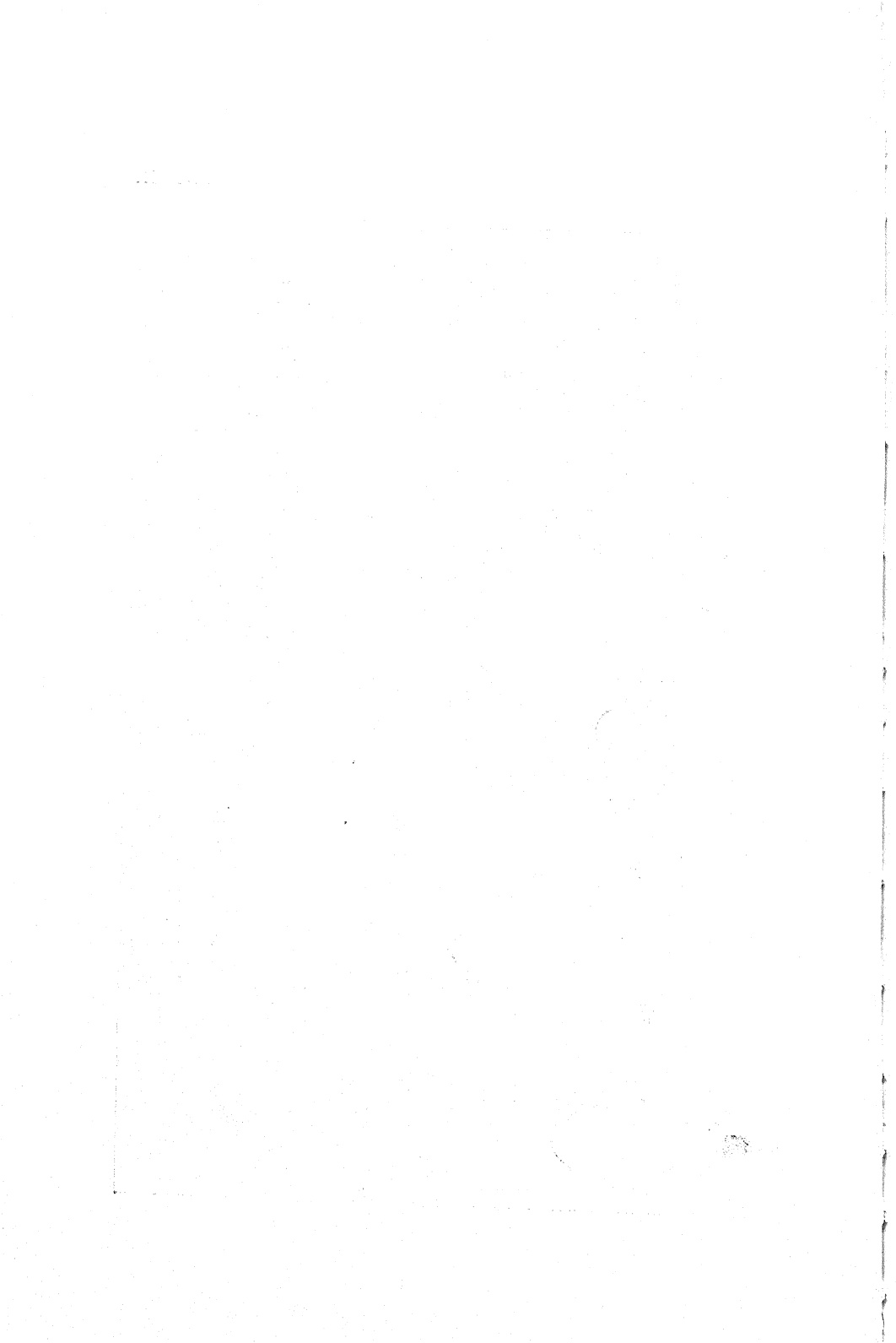


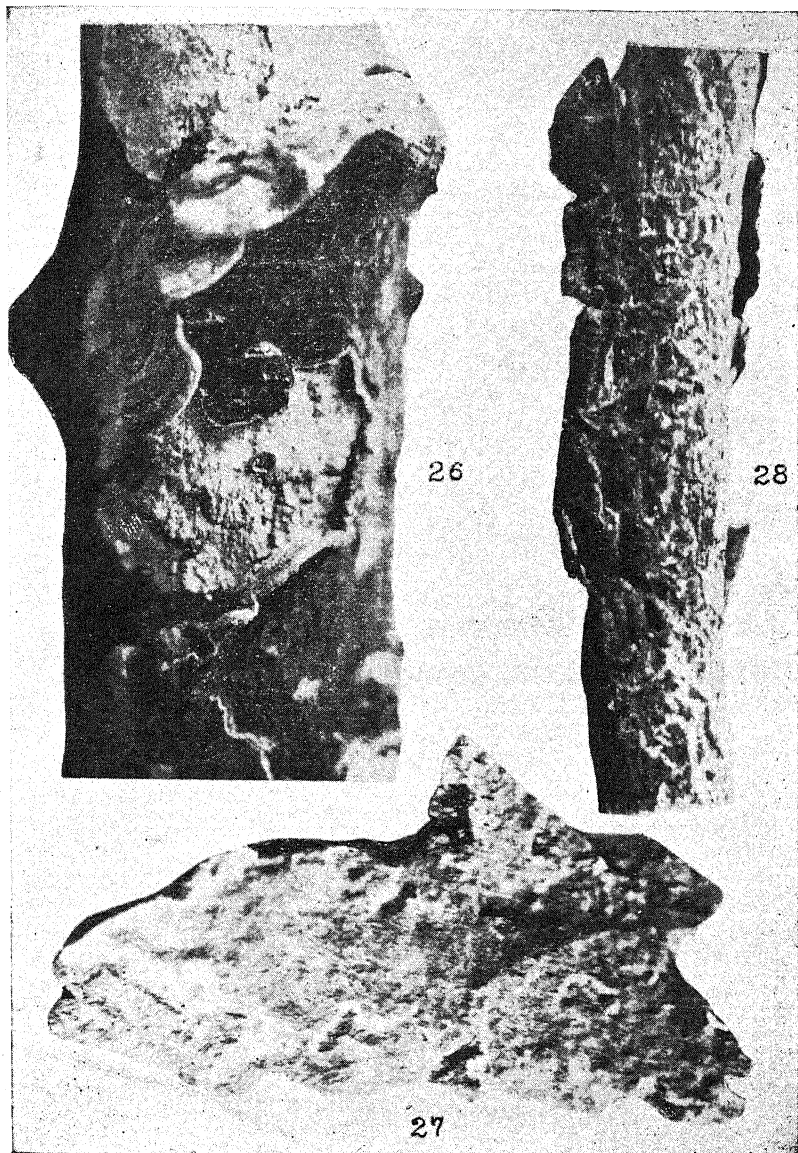
Plate II

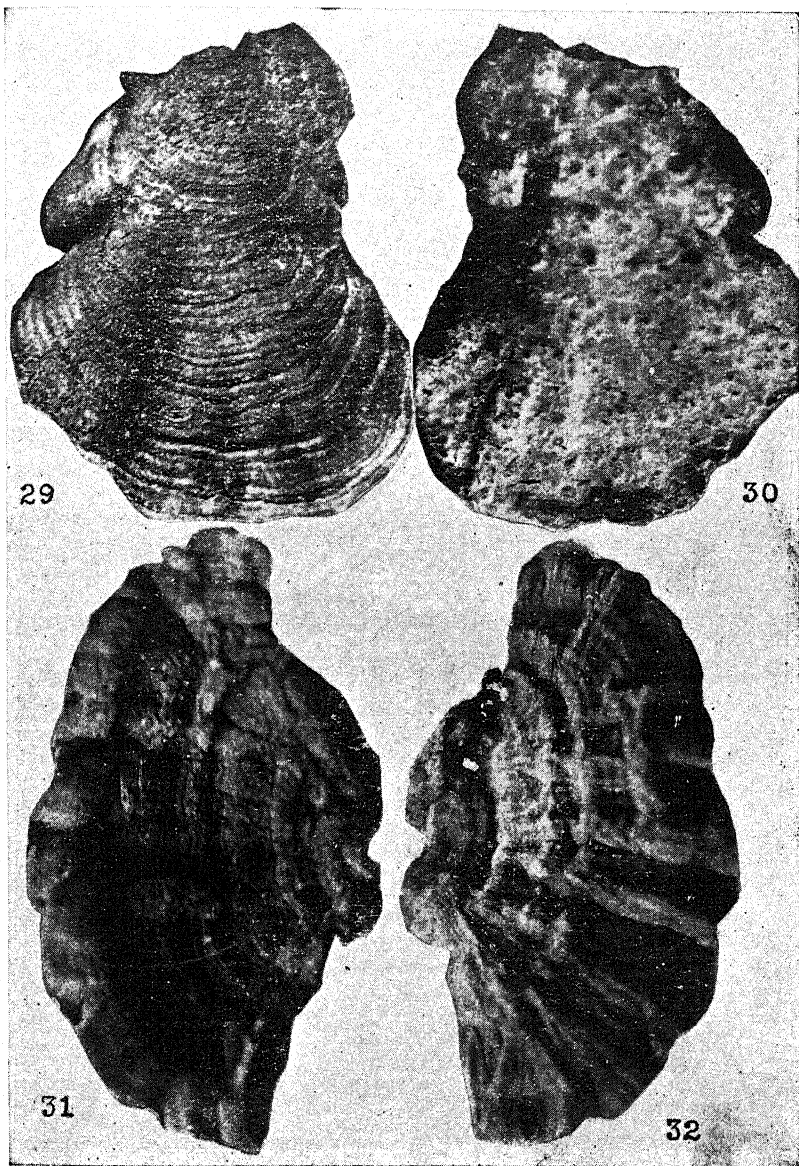


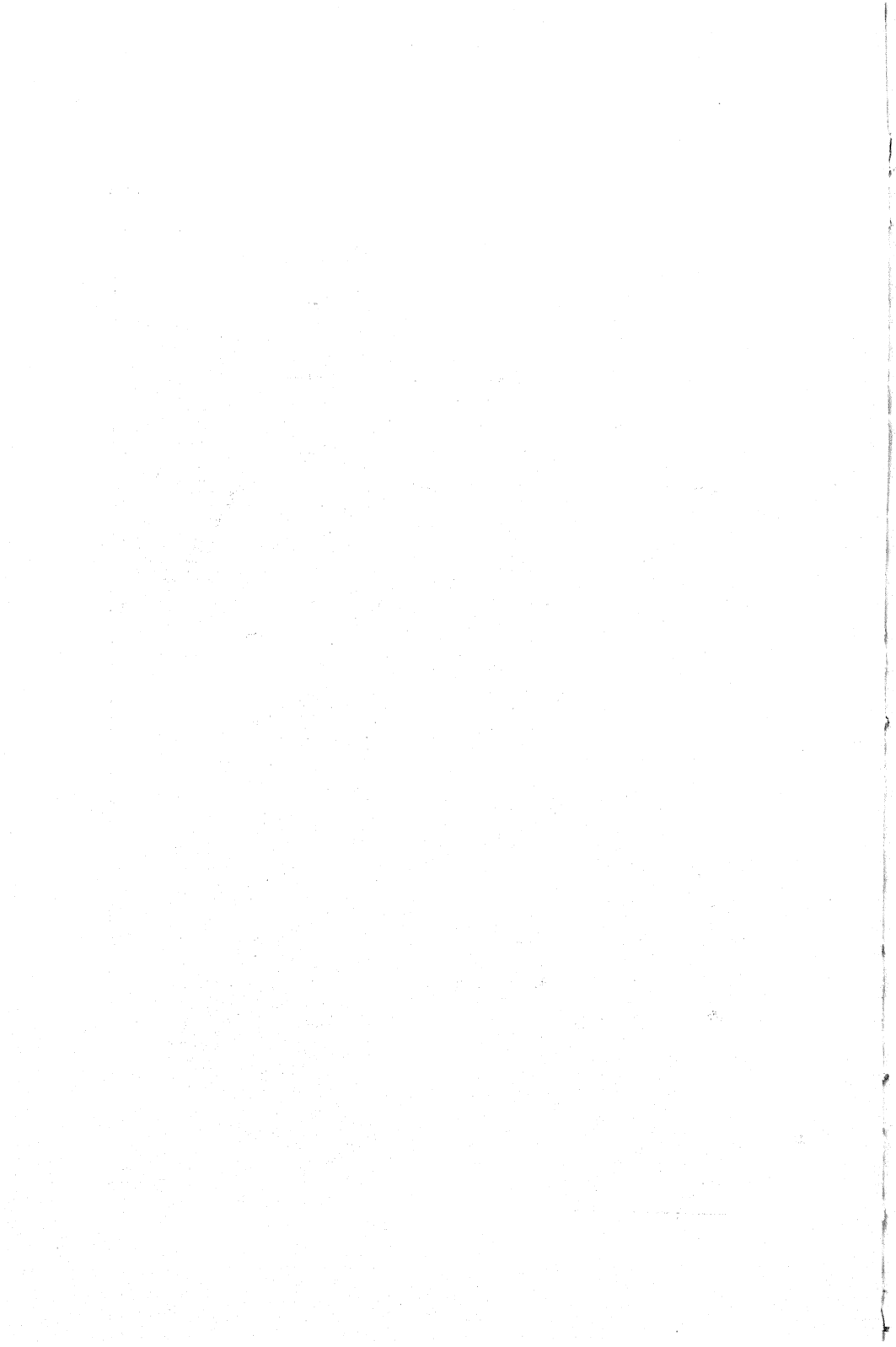
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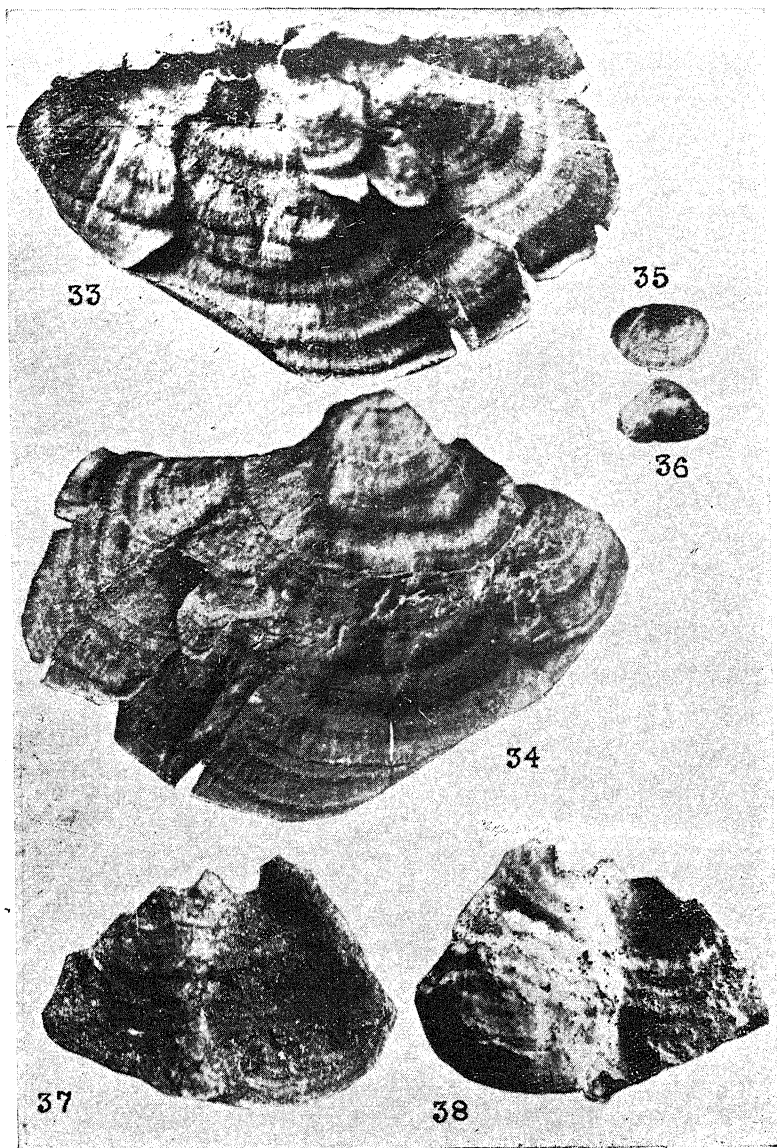


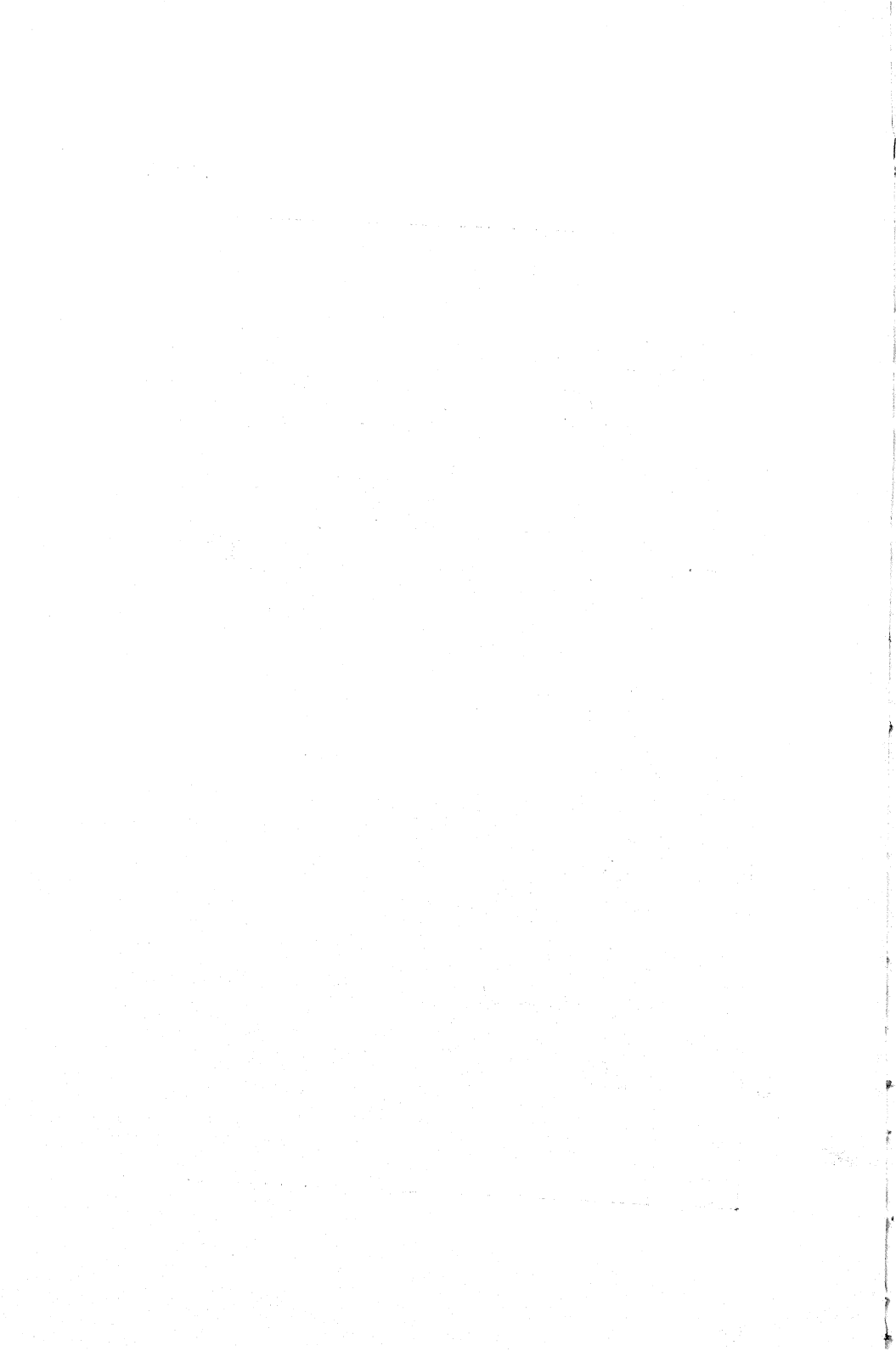


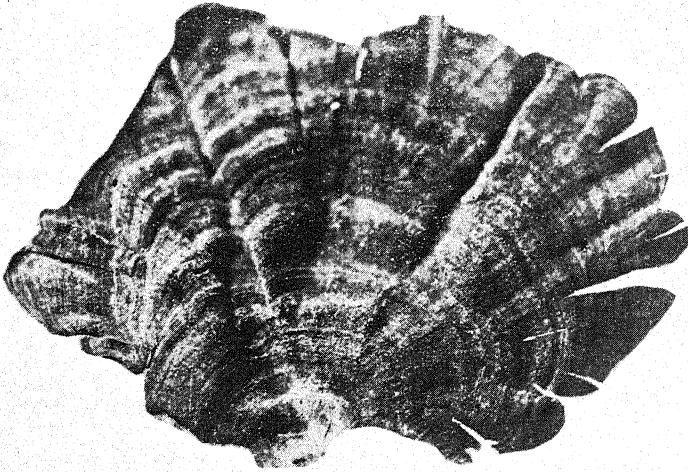




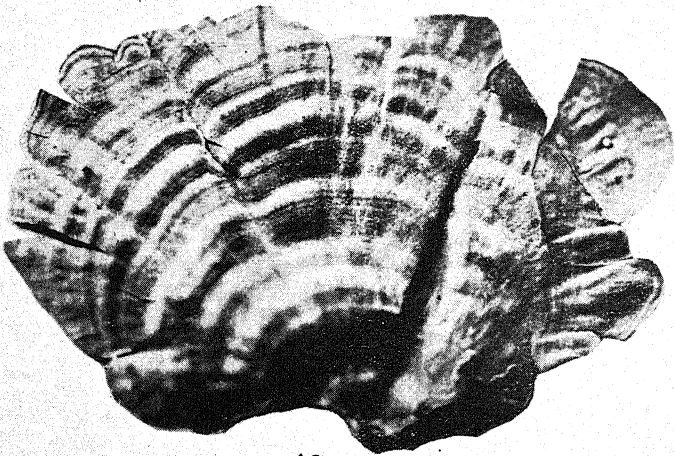




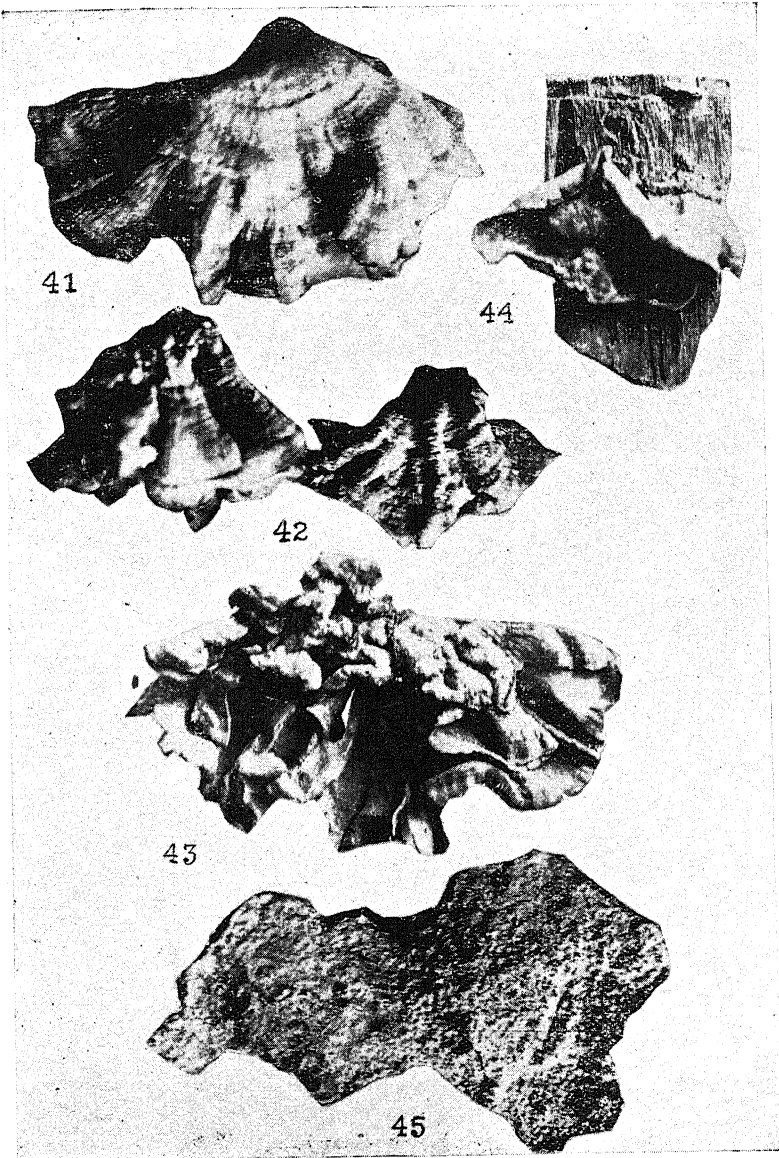


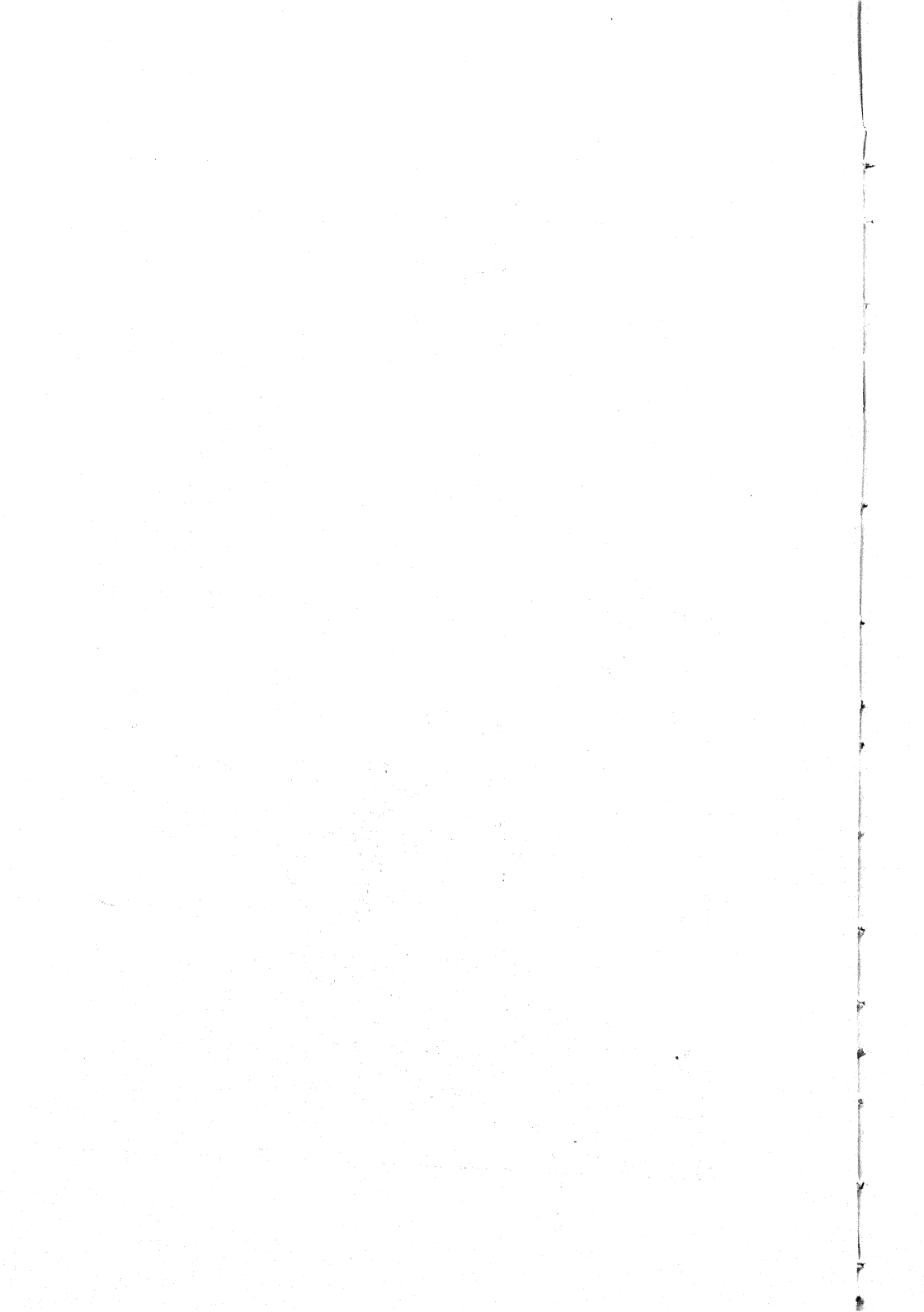


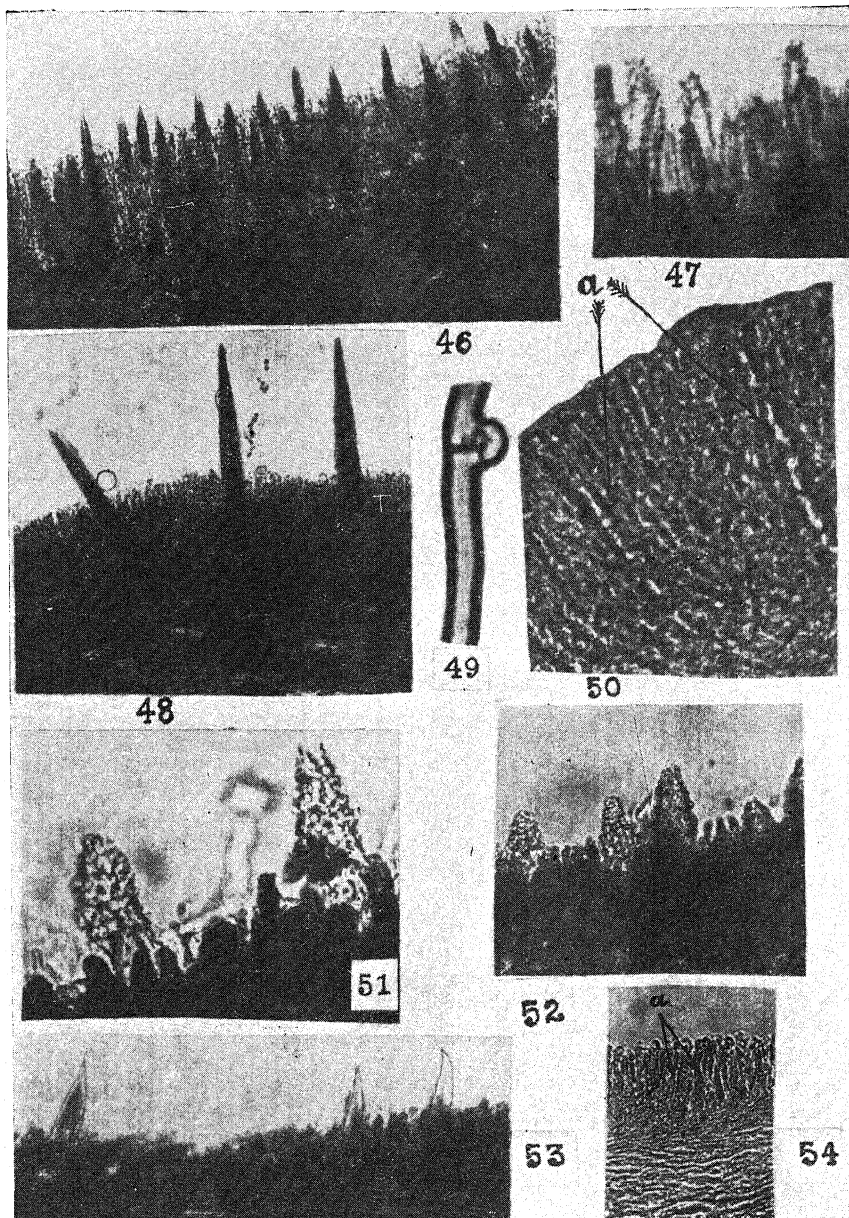
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STUDIES IN THE FAMILY ALISMACEÆ.

I. LIMNOPHYTON OBTUSIFOLIUM, MIQ.

BY

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(Received for publication on 8th September, 1933)

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Introduction

Several plants of the family *Alismaceæ* have been worked out by various investigators. In some an eight-nucleate embryo sac has been described and in others a six-nucleate one has been found. A single species, *Alisma plantago*, has been investigated by no less than six investigators, all giving different accounts of the embryo sac development. My interest in the family arose due to the reported inconsistency of the embryo sac development, and in this paper, which is the first of the series, I wish to communicate the results of my work on *Limnophyton obtusifolium*.*

Previous Work

The development of the female gametophyte has been investigated so far in five genera of the family. A short summary of the work done is given below:—

ALISMA PLANTAGO:—WARD (32) in 1880 mentioned that the megaspore mother cell divides into two cells, of which the upper divides once and the two cells thus formed degenerate. The nucleus of the lower cell divides thrice and

*A preliminary report of this investigation has already appeared elsewhere (JOHRI, 12).

produces a typical eight-nucleate embryo sac. FISCHER* in the same year noted that the upper cell remains undivided and degenerates while the lower behaves as described by WARD. SCHAFFNER (23) in 1896 found that a wall cell is cut off by the archesporial cell and the development of the female gametophyte is of the "Lilium type", resulting in an eight-nucleate embryo sac. NITZSCHKE* in 1914 found that the megaspore mother cell produces four free nuclei of which three are cut off at the top by a wall and degenerate, the remaining nucleus divides thrice and produces an eight-nucleate embryo sac. Sometimes NITZSCHKE found only two nuclei at the chalazal end of the embryo sac instead of the usual four. DAHLGREN (7) who worked on the same plant in 1928, has shown that all previous accounts of the development of the female gametophyte in this plant are erroneous. He finds that there is a hypodermal megaspore mother cell which divides into two cells of which the upper soon degenerates. The lower divides twice to form four nuclei, two at the micropylar end and two at the chalazal end. The two chalazal nuclei do not divide further, but the two micropylar nuclei divide once to produce four nuclei, which give rise to the usual egg-apparatus and the upper polar. Of the two nuclei at the chalazal end, one functions as the lower polar nucleus and the other degenerates. The mature embryo sac is thus six-nucleate.

SAGITTARIA:—Two species have been worked out, *S. variabilis*† and *S. lancifolia*. SCHAFFNER (24) in 1897 worked out the life history of the first named species. He could not see the earlier stages in megasporogenesis but reports that an eight-nucleate embryo sac is formed in the end. The first division of the primary endosperm nucleus divides the embryo sac into a small chalazal and a larger micropylar chamber; the nucleus of the former may divide once or twice and the resulting nuclei then degenerate, while in the micropylar chamber several free nuclei are formed. COOK (5) in 1907 reported that in *S. lancifolia* the development of the female gametophyte and embryo is quite similar to that in *S. latifolia* except that the antipodal cells are ephemeral. LOHAMMAR (15) states that root tips of *S. natans* Pallas and *S. sagittifolia* L. have 22 chromosomes and that no cytological difference was observed between the two species.

ECHINODORUS RANUNCULOIDES:—DAHLGREN (7) investigated this species in 1928 and found that the megaspore mother cell after the first reduction division gives rise to two cells of which the upper degenerates, and the lower gives rise to a two-nucleate embryo sac as in *Alisma plantago*, described by him in the same paper. The primary chalazal nucleus usually does not divide; the micropylar nucleus divides twice and produces four nuclei. Thus the mature embryo sac has only five nuclei. In some cases when the primary chalazal nucleus also divides once, the resulting embryo sac is six-nucleate.

DAMASONIUM ALISMA AND ELISMA NATANS:—As investigated by DAHLGREN (7) the embryo sacs are six-nucleate and the development agrees with that in *Alisma plantago*. In some cases in *Damasonium alisma* after the four-nucleate stage, one of the two chalazal nuclei also divides, resulting in a seven-nucleate embryo sac.

SALISBURY (22) has compared the Helobiales with the Ranales on the characters of flower and vegetative parts. The comparison is in particular between the *Ranunculaceæ* and the *Alismaceæ*. He thinks that there is a very close affinity between the two groups than is indicated by the present day classification.

*Quoted in DAHLGREN (7).

†This was really *Sagittaria latifolia*. The first identification was wrong and the mistake was later accepted by SCHAFFNER himself (25).

Material and Methods

Limnophyton obtusifolium has not yet been reported from Northern India though HOOKER (10) mentions that it is found in the Deccan. In the beginning some material fixed in formalin-acetic-alcohol was very kindly handed over to me in paraffin by Dr. P. Maheshwari, together with a few prepared slides. Later I fixed a large amount of material of all ages in different killing fluids. Older flowers were trimmed on two sides to facilitate infiltration.

Of all the fixatives used, Nawaschin's Fluid (McLUNG, 18, page 157) gave the best results. The usual methods of infiltration and imbedding were followed. Sections were cut 4-25 microns thick. For sticking sections to the slides a mixture of "gloy" and potassium dichromate in distilled water was used (MAHESHWARI, 16, page 220).

The slides were stained in Iron-alum Hæmatoxylin and differentiated in a saturated solution of Picric acid in distilled water (MAHESHWARI, 17). This gave exceptionally bright staining. Sections cut from material fixed in fluids containing Osmic acid, were first bleached in hydrogen peroxide. The material takes the stain so rapidly that in hot weather one minute's immersion in hæmatoxylin was sufficient.

External Morphology

The plant is an erect aquatic herb, three to four feet high. The rhizome is thick and is covered all over by numerous slender roots and the sheathing bases of petioles. All the leaves are radical. The petioles vary from 1½-2 feet in length or even more in the oldest leaves. The lamina is sagittate with a rounded apex. There are two basal divergent lobes, which are two to three nerved and are much narrower than the upper lobe, which is oblong and five nerved.

The young scape is enclosed by three bracts and is hidden among the bases of leaves. When mature, it is 3-4 feet high and bears 5-8 whorls of flowers, which are either male or hermaphrodite. Purely female flowers are absent. Both kinds of flowers are situated in the same whorl, but the lower whorls have a greater percentage of bisexual flowers. As one proceeds from the base to the apex the number of male flowers increases till at the top there may be only a few or no hermaphrodite flowers.

There are 12-15 flowers in the lower whorls and 5-10 in the upper ones. Compound inflorescences are also found occasionally due to the growth of lateral inflorescences from lower whorls of the main peduncle.

The male as well as the bisexual flowers have three green persistent sepals and three white caducous petals. There are six shortly

stalked stamens in both kinds of flowers. SYKES (30) found rudimentary ovules in the male flowers of *Sagittaria montevidensis*, but I could not detect any such case in *Limnophyton*.

Microsporogenesis

The anther is first a mass of richly protoplasmic meristematic cells and presents a circular appearance in cross section (fig. 18). Later it becomes slightly oval and two-lobed (fig. 19) and then four-lobed (fig. 20). Simultaneously with the appearance of these lobes, there appears in each corner a group of hypodermal archesporial cells (fig. 21), distinguished from the other cells by their larger nuclei and slightly different staining reactions. As the anther grows, the four groups of archesporial cells become more and more conspicuous. Two exceptional cases were met with. In one case a two-lobed anther showed two hypodermal groups of archesporial cells (fig. 22), and in the other case an anther showed six groups of archesporial cells (fig. 23). It seems that in the former case the archesporial cells differentiated earlier but in the latter case there was a fusion of two anthers.

WARMING (quoted in GRAVES, 8) studied *Zannichellia* in 1893 but was unable to trace the origin of archesporial cells. He was, however, of opinion that they were not the products of one row of cells. CAMPBELL (3) studied *Naias* and *Zannichellia* in 1897, and he states that in the beginning it is difficult to trace the archesporial cells either in form or content and as soon as they are distinguishable there is already a group of them. CALDWELL (2) in 1899 studied *Lemna minor* and found that there is only one group of archesporial cells in each anther in very early stages. As the anther grows, this group becomes divided into two by sterilization of a layer of cells, each of these is further sub-divided into two, so that the mature anther has four groups of archesporial cells. WYLIE (33) reported in 1904 that in *Elodea canadensis* only two microsporangia are formed, each consisting of a hypodermal group of five to eight archesporial cells. GRAVES (8) in 1908 studied the origin and differentiation of archesporial cells in *Ruppia maritima*, and he found that a group of archesporial cells is formed in each lobe instead of one or two cells. JULIANO (13) found in 1931 that a plate of three to four archesporial cells differentiates in each lobe in *Monochoria vaginalis*. *Hydrilla verticillata** shows a similar mode of development of the sporogenous tissue. Thus it appears that in many monocotyledons the primary archesporial cells arise in a group instead of a single row of cells.

The peripheral sporogenous cells by periclinal divisions cut off a layer of wall cells towards the outside (fig. 21). This divides

*Dr. P. Maheshwari very kindly gave me permission to refer to his unpublished work, which is being carried out in the same laboratory.

further to form two layers, the one next to the epidermis functions as the endothecium, and the inner again divides periclinally producing the middle layer and tapetum (fig. 24). Of all these the middle layer degenerates very early and by the time the microspore mother cells are ready for the first reduction division, only its degenerated remains can be seen outside the tapetum (fig. 25). The endothecium develops the usual thickenings when the anther is mature, as in most other plants. At the time the microspore mother cells get ready for reduction, the tapetal cells take a deep stain; their nuclei enlarge and are so deeply stained that little can be made out of their internal structure (fig. 25). The cells always remain uni-nucleate (figs. 24, 25 and 31).

Figure 26 shows a microspore mother cell in synizesis. After this stage the cells round up slightly and undergo two successive divisions (figs. 27-29) resulting in an isobilateral tetrad (fig. 30). The walls of the mother cells become mucilaginous and are absorbed after which the microspores soon separate and round up (fig. 31). The exine is thin and has slight projections on its surface.

TAPETUM:—During the course of the reduction divisions the tapetal cells lose their walls, their protoplasts become more granular (fig. 25) and by the time the tetrads are formed, they show slight projections towards the interior of the anther lobe. Figure 31 shows the microspores and the irregular projections of the tapetum, which are in the process of migration in between the microspores, resulting in an amœboid periplasmodium (SCHNARF, **26**, p. 34) in which the microspores are embedded (fig. 32). CALDWELL (**2**) has made similar observations in *Lemna*, and CLAUSEN (**4**) in *Sagittaria* and *Alisma*. The nuclei of the tapetal cells, which also enter along with their protoplasts, enlarge and can be seen as deeply staining structures in between the microspores (fig. 32). A tapetal periplasmodium is common to almost all the Helobiales that have been investigated and is also found in the monocotyledons in the *Araceæ*, *Lemnaceæ* and *Commelinaceæ* (TISCHLER, **31**).

MALE GAMETOPHYTE:—The microspores are 10 microns in diameter when young, but they enlarge considerably so that the mature pollen grains are about 37 microns in diameter. Perhaps the periplasmodium performs a nutritive function, for it is gradually used up during the maturation of the microspores (figs. 32-34).

For a considerable time the nucleus of the microspore lies in the centre, but eventually it moves and takes up its position near the wall. The cytoplasm surrounding it becomes very dense (fig. 35) and the nucleus divides producing a generative and a tube nucleus which are separated by an ephemeral cell-plate (fig. 36). After this disappears, the two nuclei lie free in the cytoplasm (fig. 37). SCHÜRHOFF (**28**) has made a similar observation in *Sagittaria sagittifolia*. SCHAFFNER (**24**) did not mention any such

wall in *Sagittaria latifolia*, but it is likely that it escaped his notice due to its quick disappearance. The tube nucleus is always larger but the generative nucleus contains more chromatin and stains so deeply with Hæmatoxylin that little can be made out of its structure.

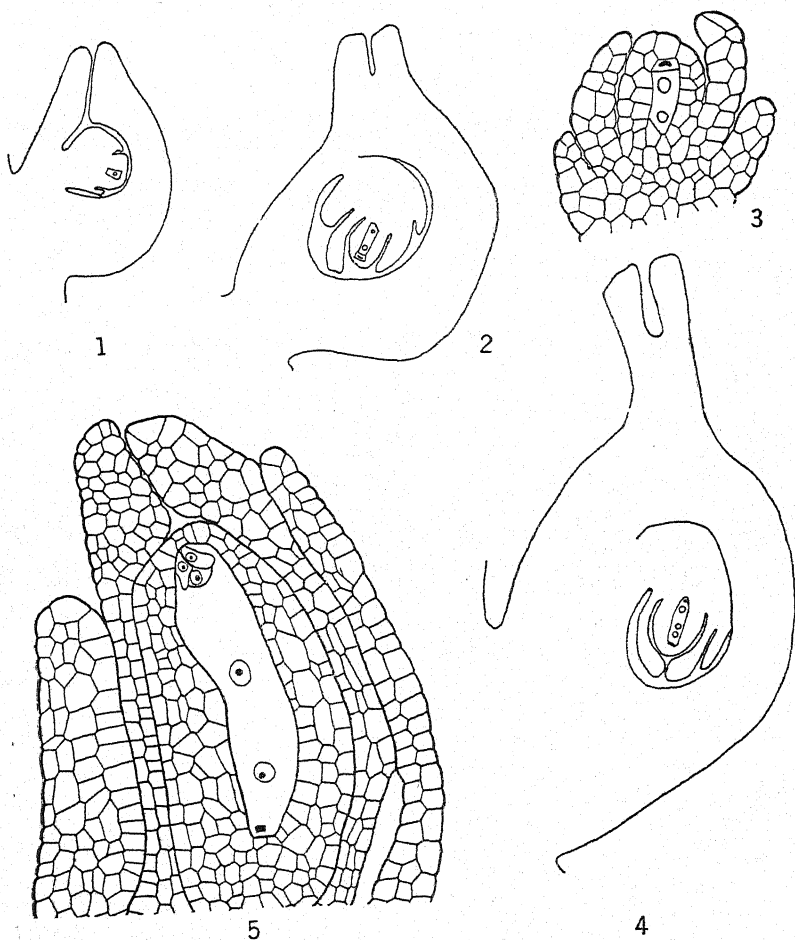
The generative nucleus divides (fig. 38) and produces two spherical male nuclei (fig. 39) which soon become spindle-shaped (fig. 39 A). The male nuclei occasionally gather some hyaline cytoplasm around them, which can be distinguished from the rest of the cytoplasm in the pollen grain (fig. 40) as mentioned by NARASIMHA MURTHY (20). But this is not a general condition. Three-nucleate pollen grains with spindle-shaped male nuclei have been reported in *Sagittaria sagittifolia* (SCHÜRHOFF, 28), and *Elisma natans* (DAHLGREN, 7). SCHAFFNER, (24) states that in *Sagittaria latifolia* the male nuclei are spherical in the mature pollen grains, and it is only after the pollen is shed that they become lenticular. The tri-nucleate pollen grains are of general occurrence in the Helobiales.

Megasporogenesis

Stages in the development of the ovule are shown in figs. 1 to 5. When mature it is anatropous and has two integuments, of which the inner is fairly well advanced even at the megaspore mother cell stage (fig. 1). This is two cells thick in the beginning (fig. 3), but due to the divisions in the cells at the apex it becomes several layered at the tip and there is a very narrow micropyle (fig. 5). The outer integument remains shorter and in later stages it fuses with the adjacent cells of the inner integument (fig. 5).

There is a single hypodermal archesporial cell (fig. 41), which functions directly as the megaspore mother cell. Figure 42 shows a megaspore mother cell in synizesis lying directly beneath the epidermis. SCHAFFNER (23) reported that in *Alisma plantago* a wall cell is cut off. He has written that "No division of this (archesporial) cell into two was observed, but at a later stage the large macrospore shows the remains of a former cell at its micropylar end, which is the tapetal cell." This was based on a wrong inference and as DAHLGREN (7) has shown no wall cell is cut off. COOK (5) could not study the origin and development of the archesporium of *Sagittaria lancifolia* and in his fig. 1 he shows an uni-nucleate embryo sac directly in contact with the nucellar epidermis. BESSEY (1) worked on *S. latifolia* in 1898 and he figured archesporial cells, which look like hypodermal megaspore mother cells. SYKES (30) saw a hypodermal cell in the rudimentary ovules of the male flowers of *Sagittaria montevidensis*, which showed all the stages of heterotypic division, but further stages were not traced. As observed by DAHLGREN (6 and 7) in *Alisma plantago*, *Echinodorus ranunculoides*, *Damasonium alisma* and *Elisma natans* and by me in *Limnophyton obtusifolium*, there is no wall cell formed.

The megaspore mother cell now passes through the prophase of the first reduction division (figs. 1 and 43), and gives rise to two cells of which the outer is much smaller (fig. 44). The nucleus of this cell stains very deeply and the cytoplasm becomes crescent-shaped. It begins to degenerate very early, though occasionally it can be detected even in later stages (fig. 4). As DAHLGREN (7)



Figs. 1-5. Stages in the development of the ovule.

- Fig. 1. L. S. ovary with megaspore mother cell. $\times 80$.
 Fig. 2. Same with a two-nucleate embryo sac with the remains of the upper dyad at the top. $\times 80$.
 Fig. 3. L. S. ovule with a two-nucleate embryo sac. $\times 170$.
 Fig. 4. L. S. ovary with a four-nucleate embryo sac. $\times 80$.
 Fig. 5. L. S. ovule with a mature embryo sac. $\times 170$.

suggests SCHAFFNER (23) mistook this cell for a wall cell in *Alisma plantago*.

The lower of the two cells divides further to give rise to the embryo sac. Figure 45 shows the first division of this cell. This mode of development in which one of the dyads resulting from the division of the megaspore mother cell gives rise to the embryo sac, is designated as the "Scilla Type" (SCHNARF, 26). Figure 46 shows the next stage in which the two nuclei are separated by a vacuole in the centre. It is to be noted that the primary chalazal nucleus is usually smaller than the micropylar.

Each of the two nuclei now undergoes a second division resulting in four nuclei, of which two lie at each pole (fig. 47). About this stage the nucellar epidermis becomes two layered at several points by periclinal divisions. Figure 46 shows one of the cells in the process of division. The embryo sac has by this time increased considerably in size. Further development is abnormal, and for the sake of convenience I will consider the fate of the micropylar and the chalazal nuclei separately.

MICROPYLAR NUCLEI:—SCHAFFNER reports that in *Alisma plantago* (23) and *Sagittaria latifolia* (24) the two nuclei divide and produce four, three of these form the usual egg-apparatus and one remains as the upper polar nucleus. This is the normal behaviour in angiosperms and is quite in accordance with the investigations of COOK (5) on *Sagittaria lancifolia*, and DAHLGREN (7) on *Alisma plantago*, *Echinodorus ranunculoides*, *Elisma natans* and *Damasonium alisma*. I have found the same thing in *Limnophyton obtusifolium* (figs. 47-49).

CHALAZAL NUCLEI:—There has been a lot of difference in the results obtained with regard to the further divisions of the nuclei in the basal part of the embryo sac. SCHAFFNER (24) found that in *Sagittaria latifolia*, as in the majority of Angiosperms, the two chalazal nuclei also divide and form four, three of which function as antipodal cells, and one as the lower polar nucleus. COOK (5) also reported three antipodal cells in *S. lancifolia*, but he says that "I was inclined to believe the antipodals in *S. lanceolata* not quite so persistent as he (SCHAFFNER) found them in *S. variabilis*." SCHAFFNER (23) reported an eight-nucleate embryo sac in *Alisma plantago*, but he states that "Often only two nuclei could be distinguished at the base of the embryo sac. But as it is quite narrow at this end, the missing ones may have been in the adjacent sections, and there indistinguishable from the nuclei of the surrounding tissue." WARD (32) FISCHER* and NITZSCHKE* have all shown four chalazal nuclei in *Alisma*. As already mentioned, DAHLGREN (7) has recently investigated four genera of the family including *Alisma*

*Quoted in DAHLGREN (7).

plantago, and he found that the two chalazal nuclei do not undergo any further divisions after the four-nucleate stage. Only one case in *Damasonium alisma* has been reported where three nuclei were seen to divide after the four-nucleate stage, and thus there was a possibility of a seven-nucleate embryo sac. In *Echinodorus ranunculoides* there are usually only five nuclei, as the primary chalazal nucleus fails to divide after the two-nucleate stage.

In *Limnophyton obtusifolium* it is occasionally seen that even at the two-nucleate stage, the primary chalazal nucleus is smaller than the micropylar (fig. 46). At the four-nucleate stage, the lowest nucleus at the chalazal end is always the smallest of all the nuclei in the embryo sac (fig. 47). After this stage the embryo sac becomes slightly curved at this end, and this nucleus is always situated in the bent basal portion (figs. 56, 59 and 60). It takes a very deep stain and may persist even up to the time of fertilization of the embryo sac or slightly later, though it usually degenerates earlier (figs. 57-58). The second nucleus at the chalazal end functions as the lower polar nucleus. The mature embryo sac is thus six-nucleate.

In some cases three or even all the four nuclei may divide, resulting in a seven- or eight-nucleate embryo sac. Out of 100 young embryo sacs, which were carefully studied, 15 were seven-nucleate, 5 eight-nucleate and all the rest six-nucleate. Figure 50 shows one of the chalazal nuclei in metaphase, while the lowest nucleus is still undivided. The two spindles in the upper part of the embryo sac were in anaphase, showing that the division of the chalazal nucleus is belated. Figure 51 shows the chalazal region of a seven-nucleate embryo sac. In rare cases both the chalazal nuclei may divide (fig. 52) and result in four nuclei at the antipodal end (fig. 53). It is also possible that occasionally the lowest nucleus undergoes a fragmentation (figs. 54-55) to produce two or three small nuclei, resulting in a seven or eight-nucleate embryo sac.

I feel convinced that there is a considerable variation in the embryo sacs of this plant and this may also explain, at least to a large extent, the different observations made by SCHAFFNER, NITZSCHKE and DAHLGREN on the embryo sac of *Alisma plantago*. The prevailing condition, however, is the six-nucleate one, as brought out by me in this paper and as found out by DAHLGREN in four other genera. NARASIMHA MURTHY (20) has mentioned an eight-nucleate embryo sac in *Limnophyton obtusifolium*, but in view of my observations this occurs only in exceptional cases.

It seems reasonable to conclude that in the Alismaceæ there is a tendency towards reduction in the number of mitoses in the basal part and this is most pronounced in *Echinodorus*, where the primary chalazal nucleus does not divide at all. This is carried still further in the *Onagraceæ*, where only four micropylar nuclei are present and here are no chalazal nuclei whatever (ISHIKAWA, 11).

A similar mode of development has been described for a few other angiosperms. MODILEWSKI (19) found this in *Neottia nidus ovis*. In the allied family *Butomaceae* two plants have been investigated — *Butomus umbellatus* and *Limnocharis emarginata*. WARD (32) observed in the first named plant that the megaspore nucleus divides twice producing a four-nucleate embryo sac. The micropylar nuclei always divide once and produce four while the antipodal nuclei may or may not divide, thus resulting in a six- or eight-nucleate embryo sac. HOLMGREN (quoted in SCHNARF, 27) showed the presence of an eight-nucleate embryo sac in the same plant. HALL (9) reports that in *Limnocharis emarginata* a tapetal cell is cut off by the archesporial cell, but it soon degenerates. The nucleus of the lower cell divides and produces a five-nucleate embryo sac. The lower polar is missing and the primary chalazal nucleus does not divide. The micropylar group is normal. NITZSCHKE (quoted in RUTGERS, 21) has questioned the accuracy of HALL's account. He says that the primary chalazal nucleus always divides once and occasionally twice, thus producing a six-or eight-nucleate embryo sac.

ORGANIZATION OF THE EMBRYO SAC:—The synergids are pear-shaped with a large vacuole in the broad basal part and the nucleus with the "filiform apparatus of SCHACHT" in the upper part (figs. 48-49). The egg nucleus lies in the lower part of the egg which protrudes below the synergids (fig. 49). One of the synergids persists for a long time even after the fertilization of the egg (figs. 56-58 and 60). The upper polar nucleus which is the largest of all the nuclei in the embryo sac moves downwards to meet the lower polar somewhere in the middle of the embryo sac. The fusion nucleus descends down to the lower part of the embryo sac.

Pollination and Fertilization

Pollination may be brought about occasionally by gravity as most of the male flowers are situated in the upper whorls of an inflorescence. Both kinds of flowers have very well developed nectaries, whose presence suggests pollination by insects. Self-pollination is unlikely as the flowers are highly protandrous and the pollen may be ripe while the ovules still show only two-nucleate embryo sacs.

The micropyle is very narrow and almost invisible. The egg is fertilized earlier than the fusion nucleus. In fig. 56 the male nucleus was sticking to one side of the fusion nucleus but the egg had already been fertilized.

Endosperm

SCHAFFNER (24) reported that in *Sagittaria latifolia* the first mitosis of the primary endosperm nucleus is followed by wall formation, resulting in a small chalazal chamber and a larger micropylar chamber. The nucleus in the latter undergoes many free

nuclear divisions, but the other nucleus divides only once or twice and the resulting nuclei degenerate. In *Alisma plantago* the first division of the primary endosperm nucleus takes place in the middle of the embryo sac, and no wall is formed. A free nuclear endosperm is also found in *Elisma* and *Damasonium* (DAHLGREN, 7).

In *Limnophyton* the fusion nucleus gathers cytoplasm around it even before fertilization (fig. 56), and becomes very conspicuous immediately after fertilization has occurred. In one flower all the stages from the applying of the male nucleus, its fusion and even the division were seen. This division, which always precedes the division of the egg, results in the formation of two nuclei separated by an ephemeral cell-plate (fig. 57). Figure 58 represents a later stage showing a small chalazal chamber and a larger micropylar chamber. The nucleus of the former undergoes only one or two divisions. The nuclei thus formed degenerate very early (figs. 59-60). The nucleus in the micropylar chamber undergoes several free nuclear divisions, and the endosperm nuclei surround the developing embryo (fig. 60). At this stage the embryo sac becomes doubled up on itself. A little later, the endosperm becomes cellular near the periphery of the embryo sac but remains free nuclear in the centre. The endosperm is very much reduced due to the huge size of the horseshoe like embryo.

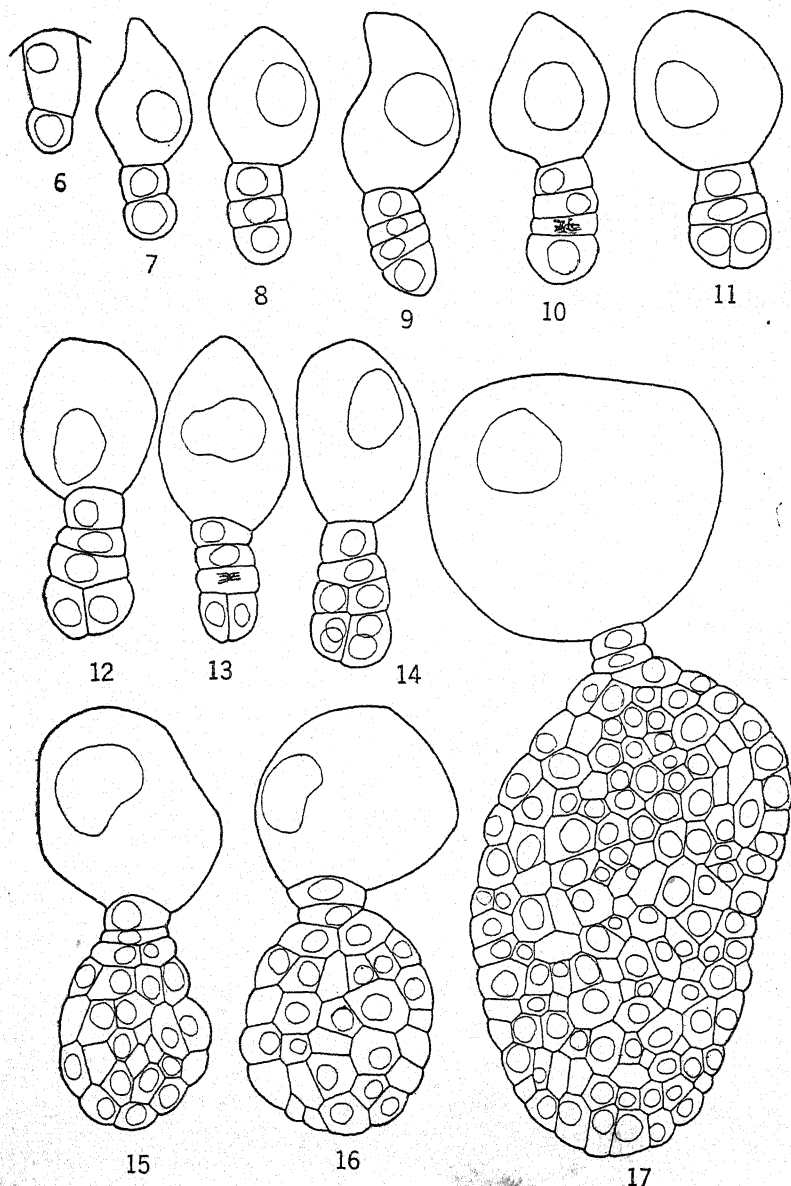
Embryogeny

After fertilization the egg elongates considerably, and the nucleus at this stage is large and conspicuous with a prominent nucleolus (figs. 56-58), one of the synergids persists but the other degenerates. The first division in the oospore is always transverse, dividing the egg into a large basal and a small terminal embryo cell (figs. 6 and 59). The basal cell does not undergo any further divisions, and all the subsequent growth of the embryo is entirely due to the division of the terminal cell. This agrees with the statement of SCHAFFNER (23) who also found that basal cell undergoes no divisions in *Alisma plantago*. The recent works of LEMESLE (14) on *Damasonium stellatum* and of SOUÈGES (29) on *Sagittaria sagittifolia* are essentially similar.

The second division takes place after the terminal cell has elongated, and results in the formation of two cells (fig. 7). Whether the next division took place in the terminal cell, as SCHAFFNER described for *Alisma*, or in the middle cell could not positively be determined. The basal cell begins to enlarge almost as soon as it is formed, and its nucleus also increases in size, finally reaching enormous dimensions, but never showing any indications of division (figs. 6-17).

The pro-embryo at this stage consists of a row of three cells and a basal cell (fig. 8). The next division occurs in the terminal cell by a vertical wall (fig. 11). Sometimes this division is delayed

so that a five-celled proembryo is formed before the division of the terminal cell (figs. 9 & 12) ; fig. 10 shows the possibility of a row of six cells before the terminal cell divides. A vertical wall is very



Figs. 6-17. Stages in the development of the embryo. $\times 284$.
For explanation see text.

soon followed in the cell adjacent to the terminal cell (figs. 13 & 14). Further divisions are in the terminal group of cells, the two central cells remaining undivided for a very long time (figs. 15-17). In older stages the embryo consists of a large basal cell, two undivided cells and a globular mass of cells in which a dermatogen can be marked out towards the periphery. The mature embryo is horse-shoe shaped.

Summary

1. There are four groups of archesporial cells in the anther. The peripheral cells cut off a layer of primary wall cells which produces an endothecium, a middle layer and a tapetum.

2. The tapetum gives rise to an amœboid periplasmodium, which begins to disappear by the time the pollen grains are 2-nucleate.

3. The microspore mother cells undergo two successive divisions and produce isobilateral tetrads.

4. The microspore nucleus divides to form a small generative and a large tube nucleus separated by an ephemeral cell-plate. The generative nucleus on division produces two spherical male nuclei, which soon become spindle shaped. Occasionally male cells have also been seen. The exine is slightly sculptured and there are three to five germ-pores.

5. The ovule is anatropous. The embryo sac is curved in later stages. There are two integuments. The micropyle is almost invisible and very narrow.

6. There is a hypodermal archesporial cell which functions directly as the megaspore mother cell. It divides into two cells of which the upper degenerates. The lower by two divisions produces a four-nucleate embryo sac. The two micropylar nuclei divide further to produce the egg apparatus and the upper polar. The chalazal nuclei usually do not divide further. Of these the upper functions as the lower polar nucleus and the lowest nucleus represents the antipodal. The mature embryo sac is six-nucleate, but in rare cases it may be seven- or eight-nucleate.

7. After the first division of the primary endosperm nucleus, an ephemeral cell-plate is laid down dividing the embryo sac into two chambers. The nucleus in the micropylar chamber produces many free nuclei while in the chalazal chamber it divides once or twice and the nuclei degenerate.

8. The development of the embryo corresponds with the *Alisma* type.

I am greatly indebted to Dr. P. Maheshwari for his constant guidance and unfailing interest in my work. I am grateful to

Prof. K. C. Mehta for encouragement and for providing the necessary laboratory facilities. Thanks are also due to Dr. K. V. O. Dahlgren for some useful suggestions and criticisms.

Post-script

Since this paper has been in the press MR. NARASIMHA MURTHY (Jour. Mysore Univ., **7**: 1933) has published some observations on the same plant (*Linnophyton obtusifolium*); DAHLGREN (Planta Archiv. für wissen. Bot., **21**: 1934) on *Sagittaria sagittifolia* and *Echinodorus macrophyllus*, and myself on *S. guayanensis* (Curr. Sci., **2**: 1934) and *S. sagittifolia* (Proc. Indian Acad. Sci., **1**: 1934-35).

With regard to NARASIMHA MURTHY's claim that the embryo sacs are eight-nucleate, I must insist that this is only a rare condition and generally only six nuclei are present. This has now been shown to be of general occurrence in the family and NARASIMHA MURTHY will have to put forth much more evidence before his conclusions can be accepted.

Another difference between his observations and mine is the precocious development of the fertilised egg reported by him, while I find that in every case the primary endosperm nucleus divides first. I am unable to suggest any explanation of this difference and regard his observations to be erroneous.

I may further point out that judging from his figs. 35 & 37, one would conclude that the nucellus is disorganised very early and the embryo sac is lined directly by the cells of the inner integument. This is again incorrect. Evidently he has mistaken the nucellus for the inner integument. The correct relations are shown in my fig. 5, p. 55.

Botany Department,

May 1935.

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Explanation of Plates

PLATE I (FIGS. 18—23).

Fig. 18. T. S. young anther, circular in outline and consisting of homogenous meristematic cells. $\times 361$.

Fig. 19. The same, slightly older stage, it has become oval and two lobed. $\times 361$.

Fig. 20. T. S. half of a four-lobed anther in which archesporial cells have begun to differentiate. $\times 361$.

Fig. 21. The same, showing one group of archesporial cells in each lobe. The peripheral layer of cells has divided to form the primary wall layer. $\times 630$.

Fig. 22. An abnormal two-lobed anther in which the archesporial cells have differentiated. $\times 175$.

Fig. 23. Another abnormal anther showing six groups of archesporial cells. $\times 175$.

PLATE II (FIGS. 24—33).

Fig. 24. T. S. one anther lobe showing the endothecium, the middle layer and the tapetum surrounding the group of sporogenous cells. $\times 630$.

Fig. 25. The same, at an older stage. The middle layer has degenerated, the tapetum has become very conspicuous and the mother cells have rounded up. The spaces represented in between the mother cells are all full of a mucilaginous substance. $\times 395$.

Fig. 26. A mother cell in synizesis. $\times 790$.

Fig. 27. The same in late anaphase of the first reduction division. $\times 790$.

Fig. 28. A dyad of microspores. $\times 790$.

Fig. 29. Second reduction division, telophase. $\times 790$.

Fig. 30. An isobilateral tetrad of microspores. $\times 790$.

Fig. 31. Part of an anther lobe in which the microspores have separated and the tapetal protoplasts show irregular projections. $\times 395$.

Fig. 32. An amœboid periplasmodium has been formed, the microspores have slightly enlarged and are embedded in the periplasmodium. The tapetal nuclei have also enlarged and are very conspicuous. $\times 395$.

Fig. 33. The periplasmodium has decreased while the microspores have enlarged still more. $\times 395$.

PLATE III (FIGS. 34—44).

Fig. 34. The periplasmodium in process of degeneration. $\times 395$.

Fig. 35. Microspore with its nucleus ready for division. $\times 630$.

Fig. 36. Pollen grain with tube and generative nuclei separated by an ephemeral cell-plate. $\times 630$.

Fig. 37. Same, later stage in which the ephemeral cell-plate has disappeared and the two nuclei are lying free in the cytoplasm. $\times 630$.

Fig. 38. Division of the generative nucleus in the pollen grain. $\times 630$.

Fig. 39. Three-nucleate pollen grain with two spherical male nuclei. $\times 630$.

Fig. 39-A. Same, the male nuclei have become spindle-shaped. $\times 630$.

Fig. 40. Same, there is a layer of hyaline cytoplasm round the male nuclei. $\times 630$.

Fig. 41. L. S. young nucellus showing a hypodermal archesporial cell. $\times 630$.

Fig. 42. Megaspore mother cell in synizesis; the nucellus also shows the appearance of the inner integument. $\times 630$.

Fig. 43. Megaspore mother cell ready for division. $\times 630$.

Fig. 44. Megaspore mother cell divided into two, the upper cell has degenerated and the lower has enlarged. $\times 630$.

PLATE IV (FIGS. 45—53).

Fig. 45. The lower cell in division. $\times 630$.

Fig. 46. A two-nucleate embryo sac with remains of the upper dyad. The nucellar epidermis has become two-layered. The primary chalazal nucleus is smaller than the micropylar. $\times 630$.

Fig. 47. A four-nucleate embryo sac. The lowest chalazal nucleus is the smallest. $\times 630$.

Fig. 48. A six-nucleate embryo sac. The upper polar is the largest of all the nuclei of the embryo sac. The synergids have begun to organise. $\times 630$.

Fig. 49. A mature embryo sac with the egg-apparatus, the two polar nuclei and a single antipodal nucleus. $\times 630$.

Fig. 50. Lower part of an embryo sac showing one of the chalazal nuclei in metaphase. $\times 630$.

Fig. 51. Lower part of a seven-nucleate embryo sac. $\times 630$.

Fig. 52. Both the chalazal nuclei dividing. $\times 630$.

Fig. 53. Lower part of an eight-nucleate embryo sac. $\times 630$.

PLATE V (FIGS. 54—60).

Fig. 54. The lowest chalazal nucleus showing signs of fragmentation. $\times 630$.

Fig. 55. The two lowest nuclei formed probably due to fragmentation. $\times 630$.

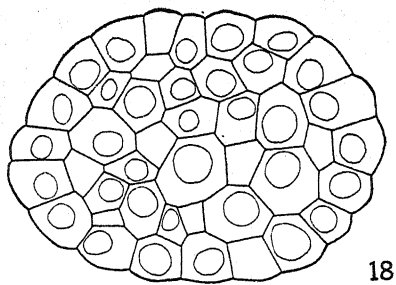
Fig. 56. A fertilised embryo sac. $\times 284$.

Fig. 57. Division of the primary endosperm nucleus has just finished and the two nuclei are separated, by an ephemeral cell-plate. $\times 284$.

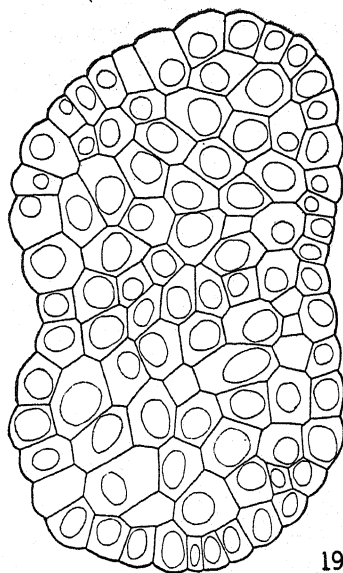
Fig. 58. Embryo sac showing the two nuclei formed from division of primary endosperm nucleus. The cell-plate has disappeared but the lower nucleus has a mass of cytoplasm around it. $\times 284$.

Fig. 59. Embryo sac with two-celled embryo. There are two endosperm nuclei in the micropylar as well as the chalazal chamber. $\times 175$.

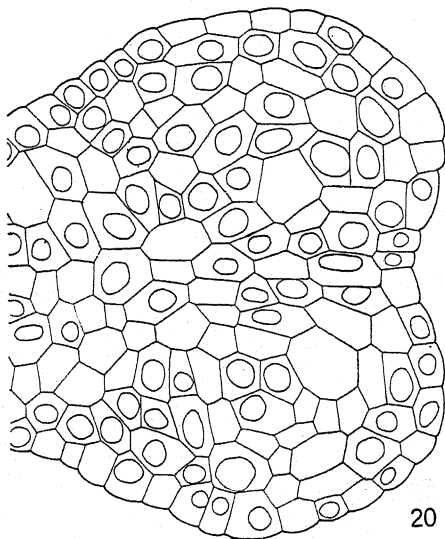
Fig. 60. A five-celled embryo surrounded by free nuclear endosperm. The chalazal chamber shows four degenerated nuclei. One synergid and the single antipodal still persist. $\times 175$.



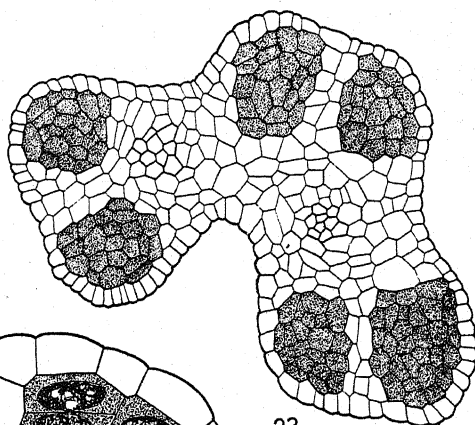
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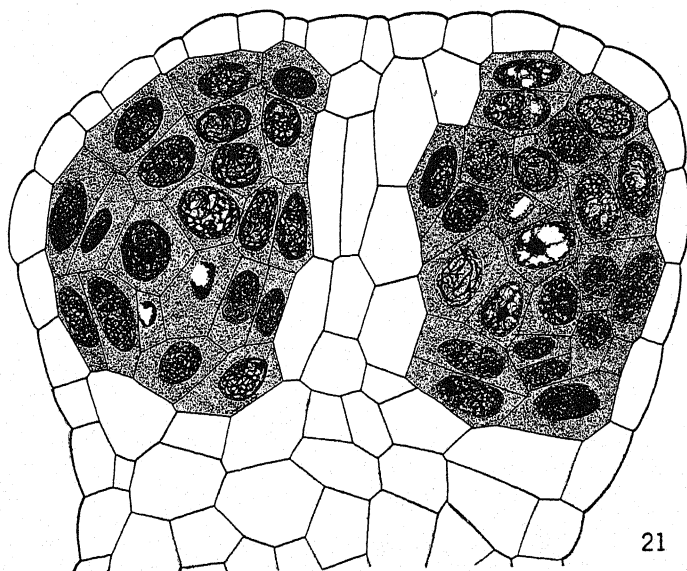
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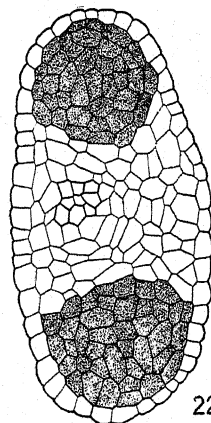
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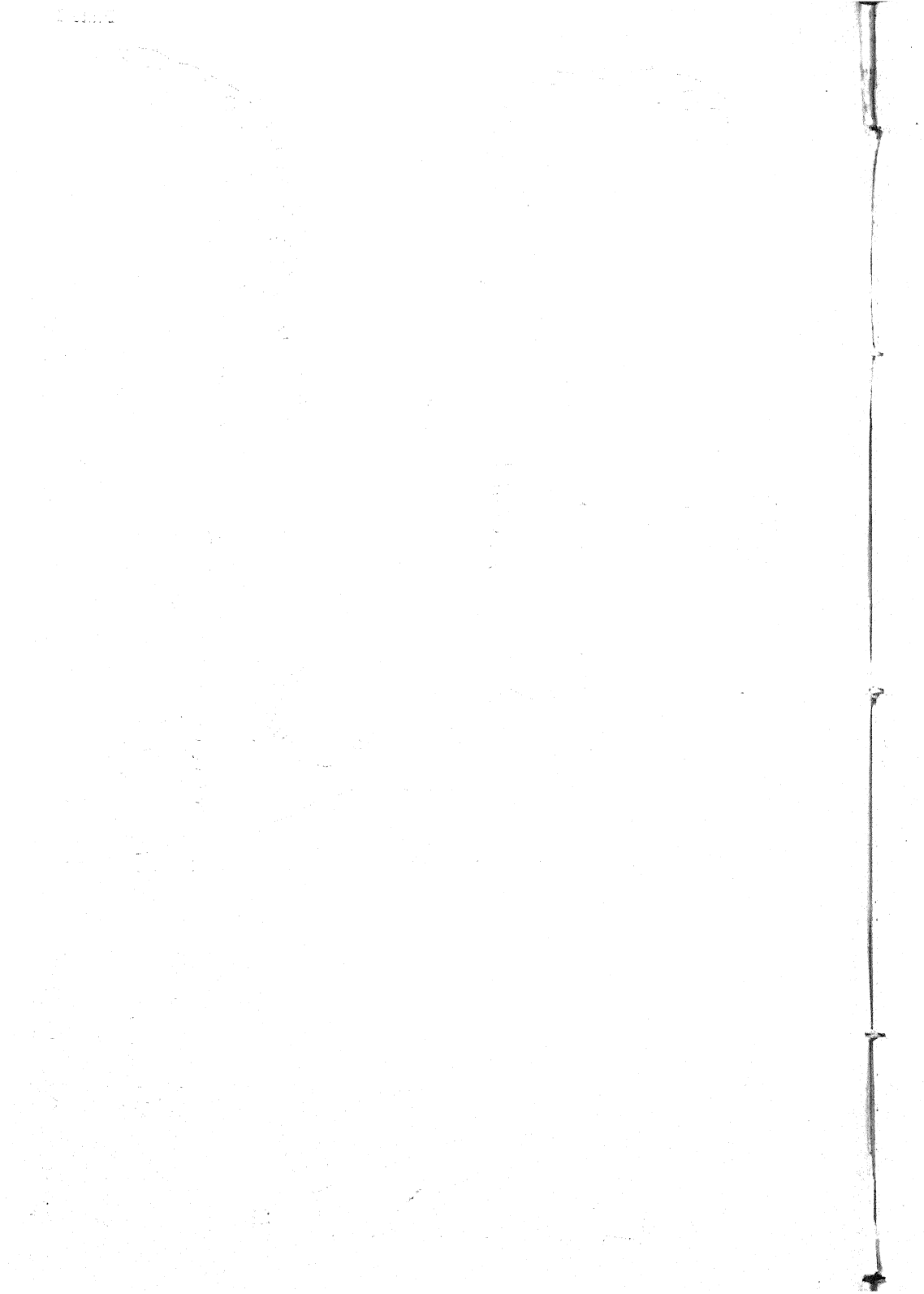
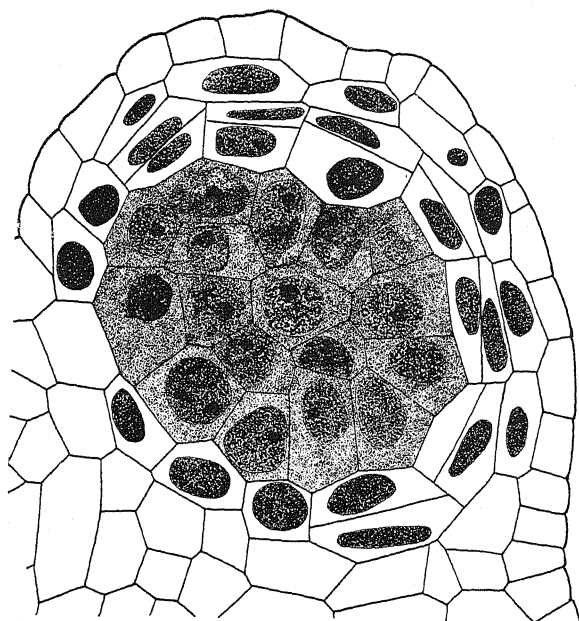
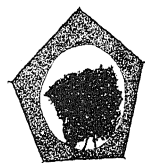


Plate II



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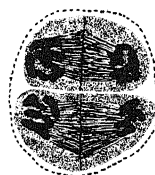
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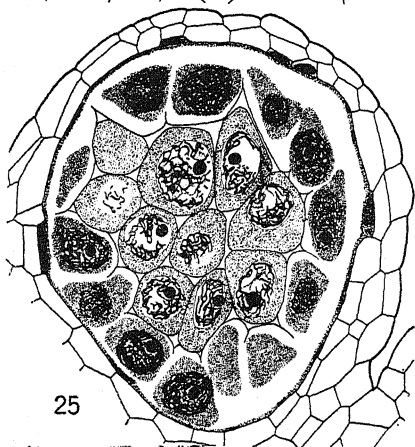
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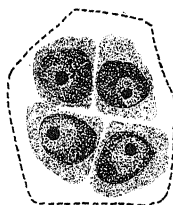
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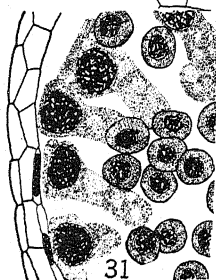
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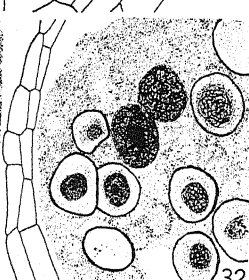
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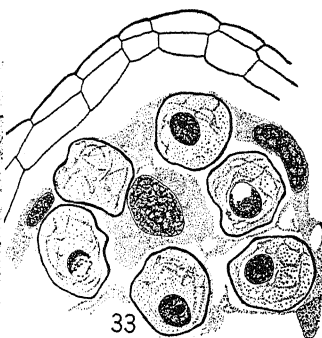
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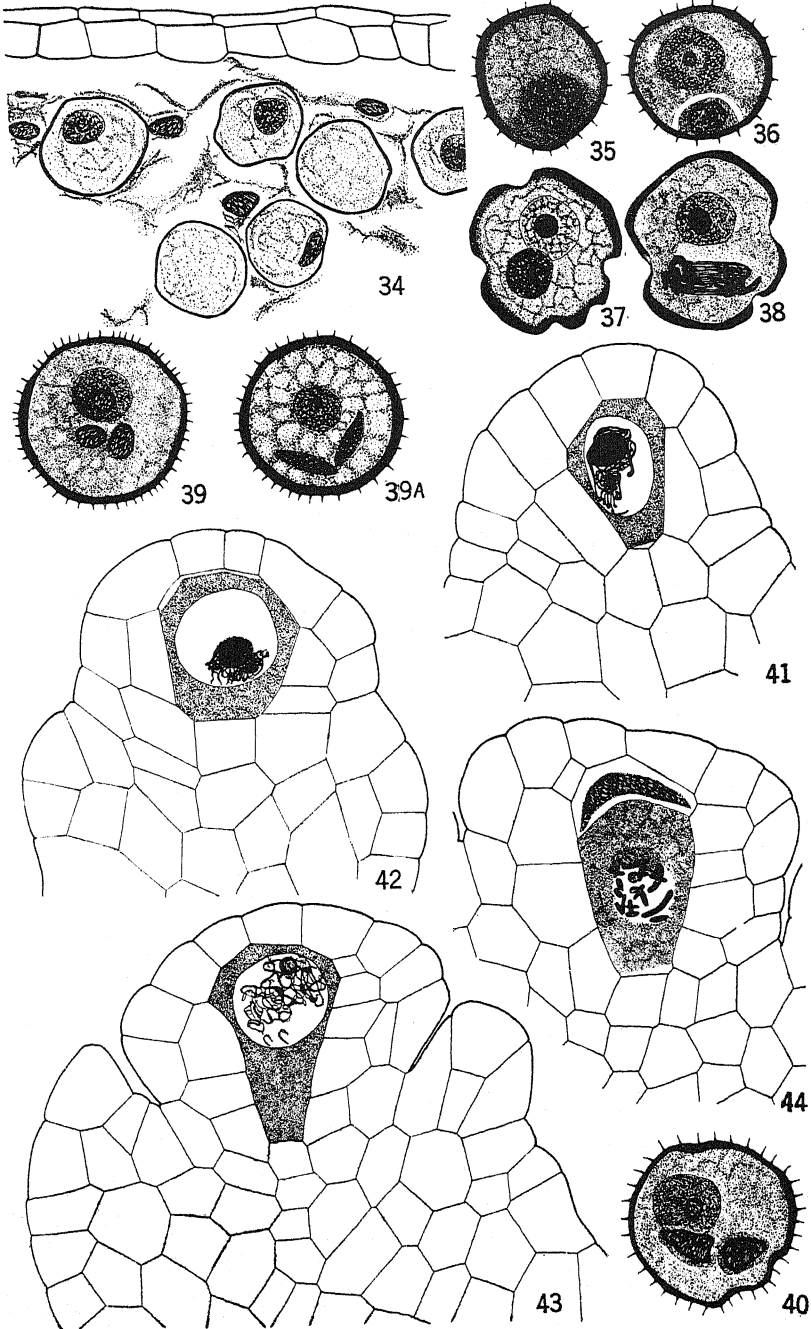
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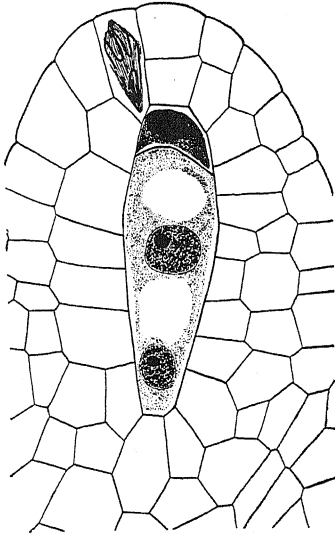
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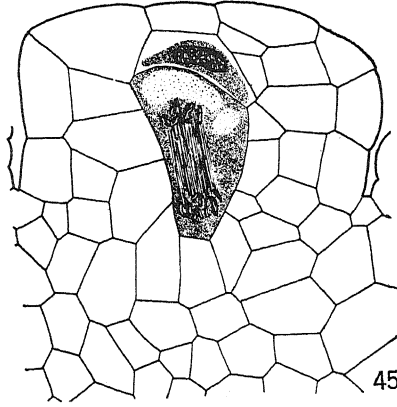
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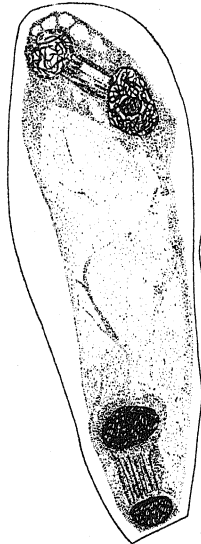




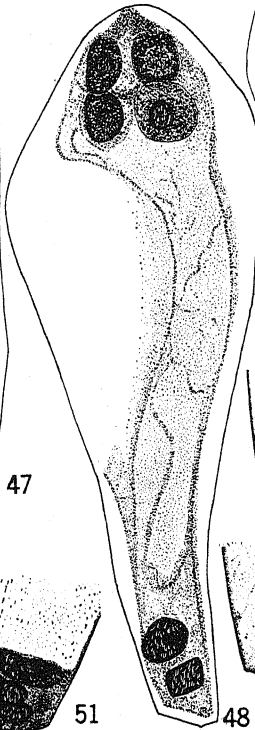
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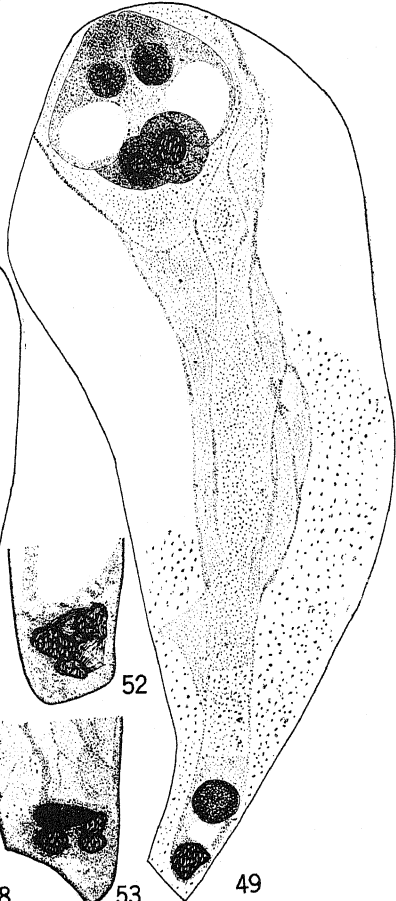
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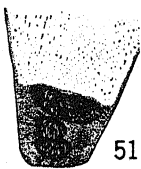
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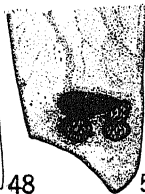
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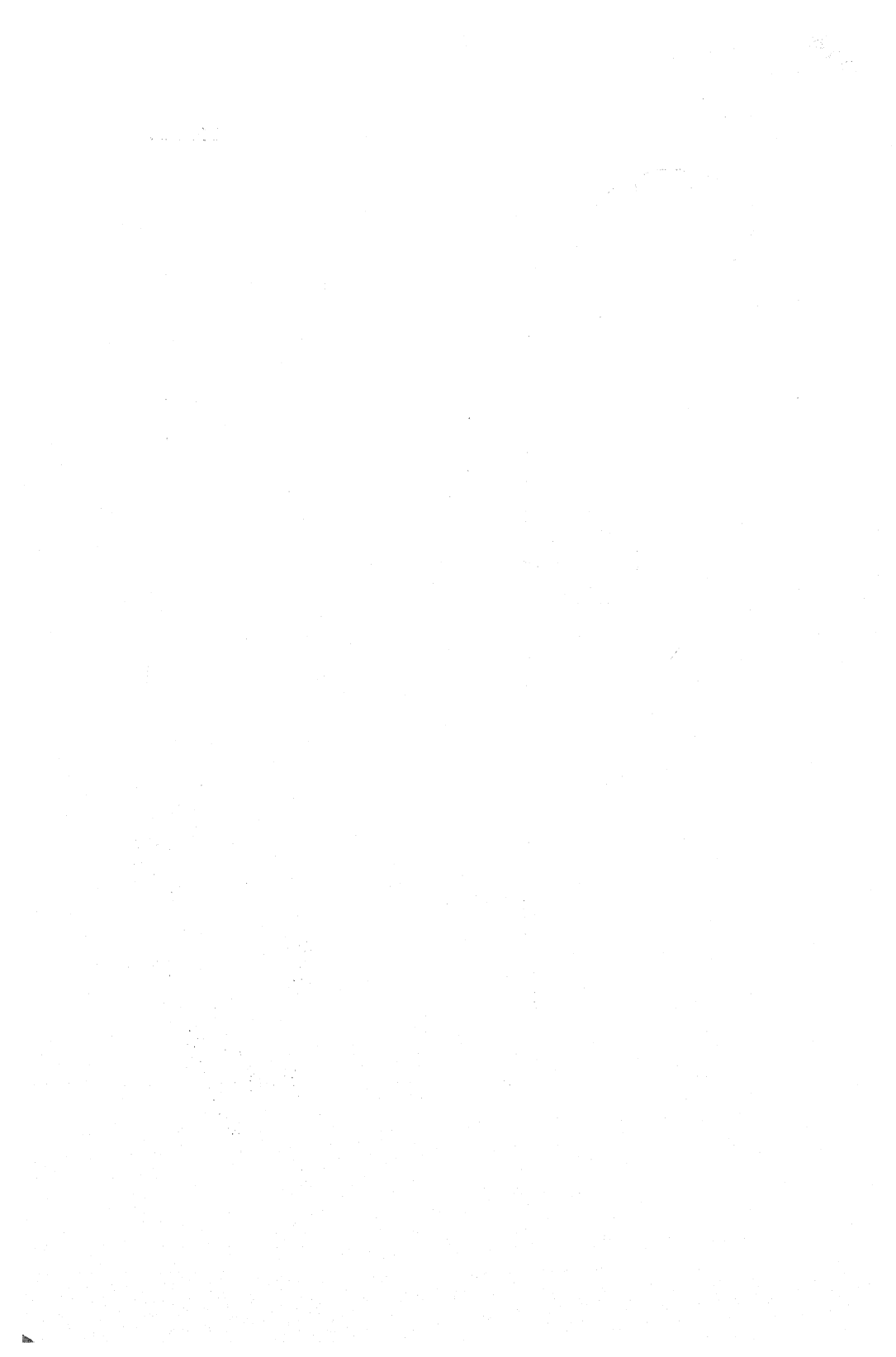
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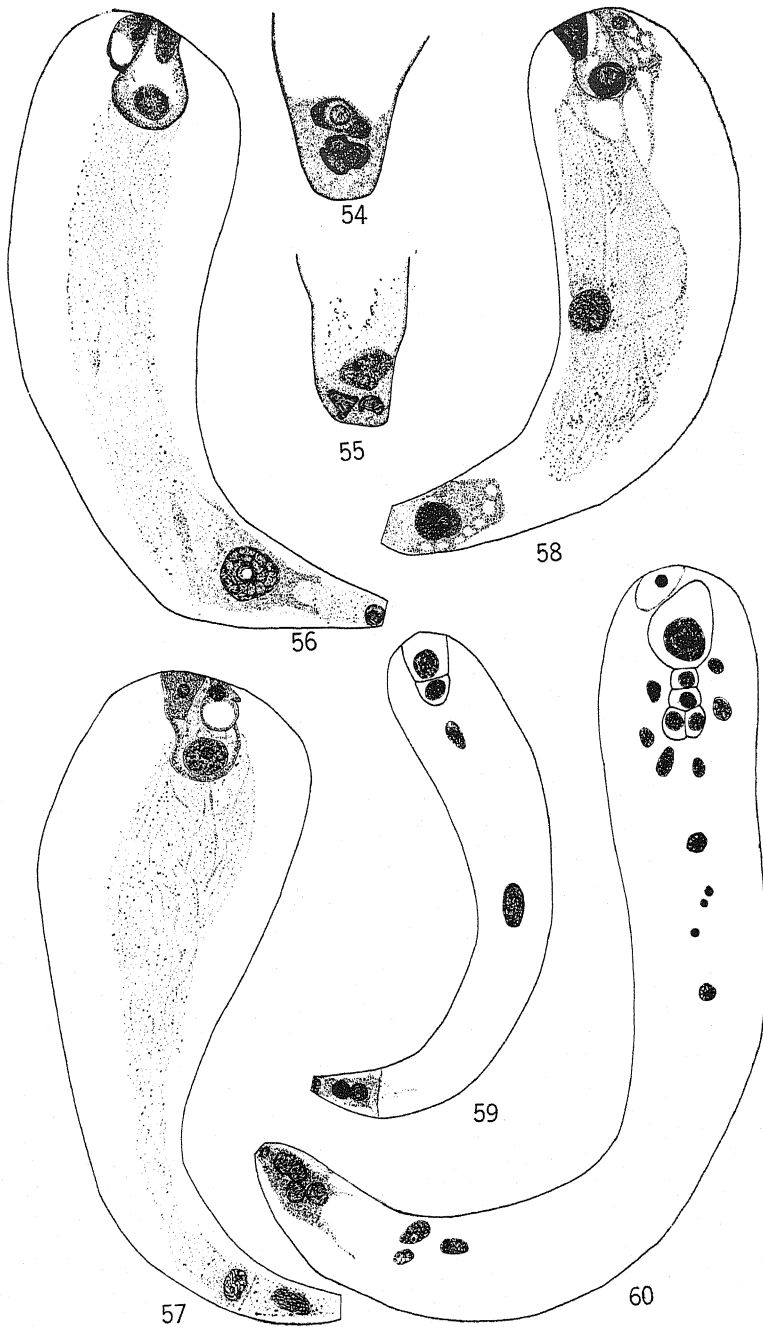


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A FURTHER NOTE ON THE IRON HÆMATOXYLIN TECHNIQUE

BY

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(Received for publication on 30th October 1933)

The writer intends to make a few supplementary remarks in connection with the interesting note by Dr. P. Maheshwari on the iron-hæmatoxylin technique published in a recent issue of this journal (Vol. XII, No. 2, p. 129).

For mordanting in connection with this method of staining, the writer, as a result of the rather extensive trial that he has given to almost all the ferric compounds that have been recommended for the purpose, finds that it is best to use the *liquor ferri sulphurici oxidati* of the German Pharmacopœia (or the corresponding *ferri sulphatis liquor* P.B.), as originally suggested by Benda, who worked out, some years before Heidenhain developed his process, the details of a hæmatoxylin technique involving the separate employment of mordant and stain. The ferric ammonium sulphate recommended for the purpose by Heidenhain is a salt which, as is well known, it is impossible to keep in a good condition, especially in hot moist climates, unless exceptional precautions are taken. The method of storage suggested by Dr. Maheshwari, that of stocking the salt in solution, is, as pointed out by him, also unsatisfactory, as precipitates of iron hydroxide rapidly form, and the solution progressively deteriorates. It is precisely this tendency of the solution to form precipitates that is responsible for the ugly "browning" of preparations treated with it, and no amount of mere washing can remove such insoluble material deposited in the substance of tissues.

The *liquor ferri* recommended by Benda, while it gives a stain identical with the Heidenhain stain in every particular, is free from the defects possessed by iron ammonium alum. The preparation in its concentrated form is convenient to store and is diluted for use with 10 to 15 volumes of water. No precipitates are formed either in the stock liquor or in the dilution made for use, which latter can be repeatedly employed for months; a tube of the diluted liquor which the writer has maintained in use since January 1932, in order to determine how long the solution will keep, is still (in October 1933) in a perfectly usable condition. The duration of the bath in the mordant can extend from a few minutes (10 or 20) up to several hours. Mordanting for a short length of time followed by a short stay in the staining bath gives a bluish black stain,

less dense and a little more transparent than the heavy black stain produced by a prolonged treatment, formerly favoured, both in the mordant and in the stain. The slides are, as usual, washed well after the treatment in the mordant and transferred to a "ripened" hæmatoxylin solution of a strength of from $\frac{1}{2}$ to 1 per cent.

The *liquor ferri* used as a mordant has the further advantage that given enough time to act, it bleaches osmicated material sufficiently well to render the blackening inconspicuous and thus obviates, in most cases, the need for a special process of bleaching such as Mayer's chlorine method or the use of hydrogen peroxide. In this respect its action is much more powerful than that of iron ammonium sulphate.

It is best to obtain the *liquor ferri* ready made from the chemist, but if there is any difficulty in the matter it may be prepared in the laboratory. 80 g. of ferrous sulphate in fresh green crystals are placed in a large wide beaker in a fume cup-board (or in the open air) and 40 cc. of water are added. 15 g. of concentrated sulphuric acid are next slowly poured in, the mixture being kept stirred. 18 g. of concentrated nitric acid are then poured in little by little; as the acid is added, the mixture froths up with the evolution of much heat and copious fumes of the noxious brown peroxide of nitrogen escape. The iron sulphate is oxidized and the persulphate is left in the form of a syrupy solution, which may be filtered, if necessary, after it cools.

With regard to the stain, the slow "ripening" of the hæmatoxylin solution by mere exposure to air is open to the obvious objection that solutions so ripened cannot be constant in their content of the active agent (hæmatein) concerned in the staining reaction; such solutions are, moreover, liable to become "over-ripened," the hæmatein becoming converted into higher oxidation products which have no staining power. The process of ripening by means of tap-water suggested by Dr. Maheshwari is interesting, but tap-water will vary enormously in its composition from place to place and only a trial can show whether the method will work in a particular locality; and in any case it is doubtful whether any but certain kinds of hæmatoxylin of American manufacture will be so quickly got ready for use. According to a note in a recent number of *Stain Technology* (Vol. VII, pp. 26 and 27) hæmatoxylin put on the market by certain American manufacturers is much more "oxydised" than the European product (which presumably means that a varying proportion of the hæmatoxylin has been converted into hæmatein) and therefore requires little or no ripening; in fact, in the opinion of the writer of the note (L. A. Margolena) "a good hæmatoxylin is ripe as soon as dissolved." But even the purer "less oxydised" sort of hæmatoxylin, which does require ripening can be got ready for use in a few minutes by the employment of H. E. Shortt's rapid method (*Ind. Jour. Med. Res.*, 1923) involving the use of

phenol. A gramme of any good brand of hæmatoxylin is dissolved in 95 cc. of distilled water by the aid of slow heat and when the solution begins to boil 5 cc. of liquefied crystals of carbolic acid are added. The solution is ready for use as soon as it is cool. Carbolic acid crystals naturally liquefy by the absorption of moisture from the air into a pink oily fluid and this can be used for the purpose mentioned. Hæmatoxylin thus ripened has a high penetrating power on account of the presence of carbolic acid and may be depended on, under all circumstances, to give a powerful stain without any possibility of failure.

With regard to the differentiating agent, picric acid, on account of the extremely slow speed at which it dissolves out the hæmatoxylin lake, has the great advantage of allowing a perfect control over the destaining process and permitting a very delicate differentiation of details to be produced. Benda originally recommended its use in combination with acid fuchsin according to the formula of van Gieson (*Deutsch. Med. Wochenschr.* 1898, xxx). In many cases, however, the acid fuchsin thus used as a counterstain gives troublesome overstained preparations which are toned down only with much difficulty. Any counterstain that may be required—if indeed it is ever necessary to have one in the case of cytological preparations of plant material—is therefore best used independently. In using picric acid for differentiation, it is a great advantage, as suggested by Tuan Hsu-Chuan, to use the iron alum solution (or the *liquor ferri* of Benda) in proper dilution for carrying out the first stages of the differentiation, which can be thus got through rapidly, and then to wash the slides well and transfer them to the picric acid for completing the differentiation; this method combines the advantages of both modes of procedure; the partial differentiation in the iron solution greatly reduces the necessary duration of the treatment in the picric acid and saves much time, while preserving all the advantages of a delicate and easily controlled differentiation provided by the picric acid during the final stages of the process. It must also be added that the iron solutions, used by themselves in a state of adequate dilution, can be made to give as slow a differentiation as may be desired. It is possible to get by the use of such weak solutions preparations differentiated as delicately as those obtained with picric acid in a considerably shorter length of time, although the control over the process will naturally have to be a little more watchful. The *liquor ferri* of Benda may be taken diluted with something like 40 or 50 volumes of water, or, if the iron ammonium alum is used, a solution of about $\frac{1}{2}$ to 1 per cent. may be employed. In any case, it is a mistake to use the same strengths of the ferric solutions for the differentiation as are found suitable for the mordanting process.

EFFECT OF ONE ORGANISM ON THE PARASITIC ACTIVITY OF ANOTHER*

BY

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(Received for publication on 18th November 1933)

Introductory

In an earlier publication dealing with the effect of one organism in reducing the parasitic activity of another (7), a detailed account of the reduction of parasitic activity of *Monilia fructigena* in the presence of *Botrytis Allii* was given. A similar effect was demonstrated for a number of other organisms and the reduced parasitism of *Monilia fructigena* shown by the mixed inocula was explained on the basis of staling phenomenon. More recently these investigations have been carried to a further stage and physiological analysis has been attempted in the case of a number of fungi, both parasitic and non-parasitic, using a variety of hosts. A considerable amount of generality has been shown so that a fuller statement of the facts is now considered necessary.

Method and Material

Experiments were carried out with batches of ten to twelve apples and three inocula placed on each apple, consisting of (a) the active fungus alone (b) the inactive fungus alone (c) mixed inoculum of active and inactive fungus. In some cases both the fungi used were parasitic. The inoculations were made in a standardised way as described by Granger and Horne (5). The concentration of the spores (where available) for each fungus was kept the same whether used separately or in the mixed inoculum. After five to seven days the amount of rot produced by various inocula was determined by weighing.

The following fungi were used during the course of this investigation:—

Botrytis cinerea.
Botrytis Allii.
Botrytis cinerea. (Lettuce)
Rhizoctonia solani.
Alternaria sp.
Penicillium sp. (Inactive)
Penicillium sp. (Active).
Monilia fructigena.
Helminthosporium sativum.

Sclerotinia libertiana.
Aspergillus niger.
Fusarium cæruleum.
Fusarium fructigenum (D).
Fusarium fructigenum (Biii).
Cunninghamella elegans.
Eidamia sp.
Sphaeropsis malorum.
Rosellinia.

*The investigation was carried on in the Plant Pathology Laboratory of the Imperial College of Science and Technology, London.

In addition *Bacillus subtilis* and *Actinomyces tricolor* were also used.

To start with, the purity of the fungi was assured by taking a single hyphal tip in each case as described by Brown (2). The cultures were maintained on potato extract agar and spores used for inoculation purposes were obtained from these cultures about three weeks old.

Newton's apples were used as they are available for the most part of the year; but from time to time other varieties were also tested.

Other hosts used were: Lemons, Turnip and Lettuce.

Experimental Results

The results of ten experiments, using various pairs of fungi are given in Table I. (In each set ten apples were inoculated.) To show the statistical significance of the differences of the amounts of attack recorded, a function t was calculated from the data as described by Fisher (4). For a series of ten observations a value of t equal to 2.23 is just significant.

TABLE I

Active Fungus.	Average Amount of Attack.	Mixed Inoc.	Average Amount of Attack.	t .
<i>Botrytis cinerea</i> .	2.45 grm.	<i>B. cin.</i> + <i>Cunn. elegans</i>	1.71 grm.	6.30
"	4.80 "	" + <i>Rhizoct. solani</i>	3.66 "	4.00
"	3.01 "	" + <i>F. caeruleum</i>	2.12 "	4.70
"	5.33 "	" + <i>Alternaria sp.</i>	3.49 "	5.00
"	6.58 "	" + <i>Helm. sativum</i>	5.68 "	3.20
"	3.25 "	" + <i>Eidamia sp.</i>	1.84 "	7.80
"	2.77 "	" + <i>Penicillium sp.</i> (inactive)	1.80 "	2.49
"	8.33 "	" + <i>Act. tricolor</i>	6.52 "	3.35
"	6.87 "	" + <i>Bac. subtilis</i>	4.43 "	4.48
"	2.60 "	" + <i>B. Allii</i>	0.90 "	4.70

All the results shown above are very highly significant.

The same type of experiment was repeated using lettuce leaves as host. Lettuce leaves were kept under sterile conditions in petri dishes containing moist filter papers in the lids. For the sake of comparison two inoculations were made on each leaf, one on either end. The inoculations were made by pricking with a needle and placing a drop of spore suspension, on the injured surface. After the attack had proceeded for five days the measurements of the rotted portion were taken. In any case where the two attacked areas overlapped the leaves were discarded.

The results of such an experiment are set out in Table II.

TABLE II

Lettuce leaf.	Attack <i>B. cinerea</i> (lettuce) alone.	Attack <i>B. cinerea</i> + <i>Cunninghamella</i> .
1	10.85 sq. cm.	5.40 sq. cm.
2	21.50 "	18.24 "
3	8.28 "	4.50 "
4	3.36 "	1.68 "
5	6.65 "	3.75 "
6	6.40 "	0.00 "
7	4.08 "	1.65 "
8	10.80 "	7.13 "
	12.00 "	8.74 "
Average	9.32 "	5.67 " $t = 7.60$

Controls in all cases remained sound.

The t as shown in the above table is quite large and shows clearly that the amount of rot produced is significantly greater when *Botrytis cinerea* (lettuce) is used alone.

In another series of experiments of similar type different hosts were used. The results from such a series are given in Table III.

TABLE III

Host.	No. of Inocs.	Pure Inoculum.	Average Amount of Attack.	Mixed Inoculum.	Average Amount of Attack.	t.
Lemon..	11	<i>Sclerotinia libertiana</i> .	14.75 grm.	<i>Scle. lib.</i> + <i>B. Allii</i> .	9.98 grm.	3.20
Lettuce.	9	<i>B. cinerea</i> (lettuce)	7.49sq.cm.	<i>B. cinerea</i> + <i>Rosellinia</i> .	4.56 sq. cm.	3.48
Turnip.	11	<i>Rhizoctonia solani</i> .	1.42 grm.	<i>R. solani</i> + <i>B. Allii</i> .	0.73 grm.	2.90
Turnip.	11	<i>Do.</i>	0.15 „	<i>R. solani</i> + <i>Alternaria sp.</i>	0.50 „	2.50 (negative)
Turnip.	11	<i>Do.</i>	0.45 „	<i>R. solani</i> + <i>Helminthosporium sativum</i>	0.72 „	2.20 (negative)

The first three comparisons clearly show the reduced parasitic activity in the case of the mixed inoculum, whereas the last two results indicate increased parasitic activity though the results are barely significant.

Fawcett (3) obtained similar results of increased parasitic activity while working with *Pythiacystis citrophthora* on citrus in the presence of a species of *Fusarium*. To investigate fully the results of this nature further work on these lines is required.

In another set of experiments closely allied fungi were used to find out if the reduction of parasitic activity occurred in such cases. For this two strains of a *Penicillium* species were isolated from diseased apples; one being very active and the other very weak parasite (average amount of rot produced being 8.46 and 0.82 grm. respectively).

In two sets of experiments the following type of results were obtained:—

- Apples Inoc.

1. Average Amount of Attack by *Penicillium* (active)=7.52 grm.
alone 15.
Average Amount of Attack by *Penicillium* (active+inactive)=8.02grm.
mixed
2. Average Amount of Attack by *Penicillium* (active)=15.84 grm.
alone 12.
Average Amount of Attack by *Penicillium* (active+inactive)=16.20grm.
mixed

In both the experiments the difference of attack obtained, when mixed inoculum was used against the pure inoculum, was insignificant; t being 1.9 and 0.57 respectively.

Similar results were obtained when two strains of *Fusarium fructigenum* were used. (Strains D and Biii).

In this paper physiological analysis of the effect of reduced parasitic activity has been studied in detail in the case of *Botrytis cinerea* and *Monilia fructigena* and a number of other fungi.

Reduced parasitic activity of *Botrytis cinerea* as shown in Table I, is also obtained when in mixed inoculum certain fungi are replaced by their staling products.

Spores of a number of fungi were sown in 100 cc. sterile flasks, each containing 10 cc. of apple juice. Uninoculated flasks were kept as controls. At suitable intervals the stale liquid was strained through fine muslin and centrifuged off to remove the remaining spores and mycelium. To make sure that all the fungal material had been removed a few drops of this liquid were examined under the microscope.

The spores of *Botrytis cinerea* were sown in the stale liquid thus derived and in the apple juice obtained from the control flasks. The results of eight such experiments are given in Table IV.

TABLE IV

No. Apples Inoculated	Stale liquid derived from Culture of:	Age of Stale in days	Average amount of Rot caused by <i>B. cinerea</i> sown in		t	pH	
			Apple Juice	Stale liquid		Initial.	Final.
10	<i>Cunn. elegans</i> ..	18	5.31 grm.	4.50 grm.	3.90	5.2	5.2
10	<i>Rhizoct. solani</i> .	18	7.57 "	5.53 "	3.50	5.2	5.2
10	<i>F. caeruleum</i> ..	27	3.59 "	2.57 "	3.00	5.2	5.2
1	<i>Helm. sativum</i> .	27	6.15 "	4.24 "	3.60	5.2	6.0
10	<i>Eidamia sp.</i> ..	27	4.59 "	1.85 "	4.15	5.2	5.6
10	<i>Penicillium sp.</i> (inactive)	18	4.50 "	2.86 "	3.00	5.2	5.2
10	<i>B. Allii</i> ..	24	2.18 "	1.08 "	6.88	5.2	5.2
10	<i>B. cinerea</i> ..	27	6.85 "	7.08 "	—00.0	5.2	5.6

In the first seven cases in the above table the amount of attack when *Botrytis cinerea* spores are sown in the stale liquid is significantly less. No such reduction of parasitic activity is shown when the spores of *Botrytis cinerea* are sown in the stale liquid derived from its own cultures. This result is in accordance with that obtained previously with *Monilia fructigena* (6).

Similar results were obtained when *Monilia fructigena* spores were sown in the stale liquids of a number of fungi as shown in Table V.

TABLE V

No. Apples Inoculated	Stale liquid derived from Culture of:	Age of Stale in days	Average amount of Rot caused by <i>M. fructigena</i> sown in		t	pH	
			Apple Juice	Stale liquid		Initial	Final
10	<i>Aspergillus niger</i> ..	12	18.36 grm.	12.20 grm.	4.63	5.2	5.0
10	<i>Penicillium sp.</i> (inactive) ..	12	21.31 „	1.29 „	15.20	5.2	5.0
10	<i>Helm. Sativum</i> ..	12	22.66 „	0.00 „	14.38	5.2	6.6
10	<i>F. cœruleum</i> ..	23	18.43 „	11.50 „	4.42	5.2	..
10	<i>B. cinerea</i> ..	12	28.43 „	4.66 „	19.90	5.2	..
10	<i>Sph. malorum</i> ..	12	24.32 „	11.78 „	8.57	5.2	6.8
9	<i>Cunn. elegans</i> ..	12	23.99 „	20.97 „	3.80	5.2	5.0

The reduction of parasitic activity shown in the cases of *Botrytis cinerea* and *Monilia fructigena* (Tables IV and V) is not due to the change in reaction of the stale liquid, as the maximum range of pH variation during all the experiments is from 5.0-6.8; over which both *Botrytis cinerea* and *Monilia fructigena* show very good growth.

In another series of experiments comparison was made between the amount of attack obtained when the spores of *Monilia fructigena* were sown in water and aqueous extract of young germ tubes of various fungi.

The method followed for obtaining the germ tube extract was essentially the same as described by Brown (1) for *Botrytis cinerea*. It consisted in sowing the concentrated spore suspension in fresh apple juice on horizontal glass plates and spread in thin uniform layers. Germination was allowed to proceed for forty-eight hours, germ tubes collected and washed thoroughly with distilled water and dried over calcium chloride in vacum. The dried germ tubes were finally ground into a fine powder with an equal weight of silver sand. The aqueous extract of the germ tubes when required was obtained by suspending 0.2 grammes of this powder in 3 cc. of distilled water for an hour and then centrifuged off to remove the debris. The clear extract thus obtained was used immediately for inoculation purposes.

The results obtained from three such experiments are given in Table VI. (Ten apples were inoculated for each experiment.)

TABLE VI

Inoculum.	Average Amount of Attack.	t.
<i>M. fructigena</i> spores sown in water ..	13.86 grm.	3.29
<i>M. fructigena</i> spores sown in aqueous ext. of <i>F. coeruleum</i> ..	9.66 „	
<i>M. fructigena</i> spores sown in water ..	10.25 „	4.36
<i>M. fructigena</i> spores sown in aqueous ext. <i>B. cinerea</i> ..	7.11 „	
<i>M. fructigena</i> spores sown in water ..	11.00 „	5.10
<i>M. fructigena</i> spores sown in aqueous ext. of <i>B. Allii</i> ..	7.40 „	

Experiments conducted on similar lines with *Botrytis cinerea* in place of *Monilia fructigena* gave different results though of a very interesting nature. In this connection the aqueous extract used was only derived from that of *Botrytis Allii* spores.

Table VII shows the amounts of attack obtained when *Botrytis cinerea* spores are sown in water and aqueous extract of *Botrytis Allii*.

TABLE VII

Apple No.	Amount of Attack by <i>B. cinerea</i> spores sown in		
	Water	Aqueous extract of <i>B. Allii</i>	
1	1.80 grm.	2.10 grm.	
2	2.00 „	3.50 „	
3	0.50 „	0.90 „	
4	1.00 „	1.30 „	
5	0.90 „	1.60 „	
6	1.40 „	3.60 „	
7	0.80 „	1.20 „	
8	0.90 „	1.80 „	
9	1.50 „	2.10 „	
10	1.20 „	1.70 „	
Average	1.20 „	1.98 „	t=4.00

The above table brings out clearly that the amount of attack produced when *Botrytis cinerea* spores are sown in aqueous extract of *Botrytis Allii* is significantly greater than when the spores are sown in water. Several subsequent repetitions confirmed these results.

Increased parasitic activity was also obtained when the spores of *Botrytis cinerea* were sown in its own aqueous extract.

To investigate the factor controlling such increase in parasitic activity when *Botrytis cinerea* spores are sown in the aqueous extract of *Botrytis Allii*, inoculations were carried on by sowing the spores in water and aqueous extract of *Botrytis Allii*, both heated and unheated. The object of heating the aqueous extract was to destroy the "active principle." The extract was heated at 100°C. for five minutes and the volume was made up to the original by the addition of sterile distilled water.

The results of a typical experiment are set out in Table VIII.

TABLE VIII

Apple. No.	<i>Botrytis cinerea</i> spores sown in		
	Water.	Unheated <i>B. Allii</i> Aqueous Extract	Heated <i>B. Allii</i> Aqueous Extract
1	1.70 grm.	1.90 grm.	1.20 grm.
2	8.70 "	14.70 "	10.90 "
3	4.10 "	6.10 "	2.20 "
4	6.00 "	11.00 "	4.40 "
5	5.20 "	11.40 "	7.10 "
6	2.70 "	8.60 "	7.60 "
7	10.40 "	12.90 "	12.00 "
8	10.50 "	13.80 "	13.80 "
9	8.20 "	12.40 "	9.70 "
Average	6.39 "	10.31 "	7.65 "

As shown above, the amount of attack obtained when *Botrytis cinerea* spores are sown in unheated *Botrytis Allii* aqueous extract is significantly greater than that when the spores are sown in water or heated aqueous extract. (t being 5.68 and 3.17 respectively.)

Though there is increase in the amount of attack when *Botrytis cinerea* spores are sown in heated *Botrytis Allii* aqueous extract against water yet the results are statistically insignificant. ($t = 1.71$.)

In another experiment spores of *Botrytis cinerea* were sown in its own aqueous extract, both heated and unheated and also in water for the sake of comparison. Here also results of exactly similar nature to *Botrytis Allii* were obtained. The amounts of rot produced are given in Table IX.

TABLE IX

Apple No.	<i>Botrytis cinerea</i> spores sown in		
	Water	<i>B. cin.</i> Unheated Aqueous extract.	<i>B. cin.</i> Heated Aqueous extract.
1	2.20 gm.	2.60 gm.	2.30 gm.
2	2.70 „	3.00 „	2.90 „
3	2.40 „	3.50 „	2.60 „
4	0.90 „	2.10 „	1.20 „
5	1.70 „	2.70 „	2.10 „
6	1.60 „	1.80 „	1.60 „
7	1.30 „	2.40 „	2.00 „
8	0.80 „	1.90 „	1.90 „
9	0.70 „	1.20 „	1.00 „
10	1.90 „	2.10 „	1.00 „
11	1.00 „	1.10 „	0.80 „
12	1.70 „	2.90 „	1.90 „
Average	1.58 „	2.28 „	1.78 „

In all the above cases the aqueous extract was obtained from spores which had been allowed to grow in fresh apple juice for two days. But in another experiment of similar type, for preparing the aqueous extract of *Botrytis Allii*, sterilised onion extract was used in place of apple juice.

When the spores of *Botrytis cinerea* were sown in aqueous extract of *Botrytis Allii* thus prepared, no increase in parasitic activity was obtained. This is due to the presence of certain toxic substances of onion juice which could be easily detected by their

peculiar smell. These practically disappeared on heating as the sowings made in the heated aqueous extract gave a significant increase in the amount of attack. Such inhibitory action of certain substances present in the onion juice on the growth of a number of fungi has been demonstrated by Walker (8) and Vasudeva (6).

The results obtained from such an experiment are summarised below: (10 apples were inoculated).

Average Amount of Attack when <i>B. cinerea</i> spores sown in water	..	2.22 grm.	} $t=0$ $t=3.47$
Average Amount of Attack when <i>B. cinerea</i> spores sown in Unheated <i>B. Allii</i> Aqueous Extract (prepared by using onion extract).	..	2.21 ..	
Average Amount of Attack when <i>B. cinerea</i> spores sown in Heated <i>B. Allii</i> Aqueous Extract (prepared by using onion extract).	..	3.41 ..	

It is clear from the above data that there is definite increase in the amount of attack when *Botrytis cinerea* spores are sown in heated aqueous extract against water. This can probably be explained due to the presence of certain substances in the heated aqueous extract which merely serve as an additional food material for the invading fungus.

These results are of a similar nature as given in Table VIII, column four, but are significant. This is probably due to the difference in food material in the two cases.

Attempts were made to investigate if the reduction in parasitic activity met with in mixed inoculations could be correlated with some sort of interference with the activity of the enzyme of the attacking fungus.

For this the spores of two fungi were sown together and also separately on horizontal glass plates in equal volumes. The enzyme solutions were derived, either from the liquid in which the spores of the fungus had germinated, or from an extract of the washed germinated spores. For determining the activity of various extracts potato discs of uniform thickness were used. The end point was reached when the potato discs had lost coherence. The two kinds of preparations, those obtained from the liquid of germination and those from the germ tubes themselves will be referred to as "external" and "internal enzymes" respectively.

Table X gives the times required for the decomposition of potato discs in the case of internal and external enzymes of various fungi, separately and mixed. The activities of the various preparations are inversely proportional to the time required for the decomposition of the discs.

TABLE X

Fungus.	External Enzyme.	Internal Enzyme.
<i>B. cinerea</i>	105 min. ..	195 min.
<i>B. Allii</i>	No action ..	No action.
<i>B. cin.</i> + <i>B. Allii</i>	135 min. ..	No action.
<i>Penicillium sp.</i> (inactive) ..	345 min. ..	225 min.
<i>B. cin.</i> + <i>Penicillium sp.</i> (inactive) ..	345 min. ..	285 min.

The above table clearly shows that *Botrytis Allii* and *Penicillium sp.* reduce the activity of external and internal enzymes of *Botrytis cinerea* to a marked degree when the enzymes are prepared by sowing these fungi together.

Further work along these lines is necessary.

There is no effect of the external enzymes of *Botrytis Allii* and *Penicillium sp.* on the activity of the external and internal enzymes of *Botrytis cinerea* (enzymes of each fungus being prepared by sowing the spores separately). This is clear from the data given below:—

Conc. of <i>B. cin.</i> Enzyme Ext.	Diluting Liquid.	<i>B. cin.</i> Enzyme	
		Internal.	External.
50 per cent ..	<i>B. Allii</i> Ext. Enzyme ..	90 min. ..	90 min.
50 ..	<i>Peni. sp.</i> Ext. Enzyme ..	90 min. ..	90 min.
50 ..	Apple Juice ..	90 min. ..	90 min.
50 ..	Water ..	90 min. ..	90 min.

Penicillium sp. and *Botrytis Allii* when sown in combination with *Botrytis cinerea* have a depressing effect on the general growth of the latter and the enzymes obtained from such sowings are weaker. The fact that the external enzymes produced from *Botrytis Allii* and *Penicillium sp.* have no effect on the activity of the external and internal enzymes of *Botrytis cinerea* shows that probably the reduction of activity of the enzymes as shown in Table X is due to some staling phenomenon.

Similar results of depression of parasitic activity in mixed inoculations described in the earlier part of this paper can also be explained on the basis of staling phenomenon.

The author wishes to express his sincere thanks to his esteemed Professor W. Brown for valuable criticism and interest throughout the course of this investigation.

Summary

1. The parasitic activity of *Botrytis cinerea* and a number of other fungi is reduced due to the presence of another organism in the inoculum.

2. Reduced parasitic activity is also obtained when certain fungi are sown in the stale liquid or aqueous extract of the germinating spores of other fungi.

3. There is increase in the parasitic activity of *Botrytis cinerea* when its spores are sown in its own aqueous extract or that of *Botrytis Allii*.

4. Activity of the enzyme extracts of certain fungi is reduced in mixed cultures.

5. The reduced parasitism shown by the mixed inocula is explained on the basis of staling phenomenon.

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REVIEWS

CHAMBERLAIN, C. J. *Gymnosperms, structure and evolution.*
University of Chicago Press, 1935, pp. 484. \$4.50.

The appearance of this book will be welcomed by all who have been engaged in the study and teaching of Gymnosperms. A long time has elapsed since the publication of Coulter and Chamberlain's well-known text-book on the Morphology of Gymnosperms (1910). In the revised edition (1917) only a few changes were made to avoid breaking up the continuity of the pages. During the last 2 decades so many papers have appeared on the morphology and cytology of this group, that a complete revision of the work was inevitable. This duty has been performed by the surviving author of the pair, who has presented us with a new book on the subject.

The organisation and plan of presentation still remain very nearly the same, but many new and excellent illustrations have been added which will serve as models for other workers, and a new chapter on "Alternation of Generations" has been added at the end. Throughout the book, the dominant note is that of a practical investigator, an explorer in the field and in the laboratory. Forty years' experience of such work by the author combined with a sympathetic contact with hundreds of students have enabled him to write an authoritative account and entitled him to give some advice to his younger colleagues:—

"Read, and read widely, that you may know what has already been accomplished; and read critically. But no one can read critically whose knowledge comes entirely, or even principally, from reading. You must have a first-hand knowledge of the material, must study it in the field and in the laboratory. . . . Studies in the field bring a kind of knowledge that cannot be gained in any other way; and the technical work of making slides and drawings, if properly done, affords a great stimulus to mental development."

In the opinion of the reviewer no teacher can discharge his duties satisfactorily or inspire his students, unless he has himself passed through such a stage. What is needed is not a mere recantation of phylogenies and relationships, but familiarising himself first and then his students with actual plant material. Prof. Chamberlain is a master of his subject. He has done the work admirably and placed in the hands of students a well-balanced and scholarly treatise, which is remarkable for its clarity and value. For the research worker, there are interpolated interesting, useful and suggestive sentences in every chapter. The book will hold its place for a long time and the author will be remembered with gratitude for having done his task so well.

In spite of the care that has been exercised in revising the MSS., some mistakes have crept in and several important papers on both living and fossil representatives of the group find no mention. *Crossothea* is still regarded as the male fructification of *Lyginopteris*, although opinion has now shifted to *Telangium*, or the question may at best be left an open one. On p. 303, it is mentioned that the earliest conifer, in which a hypodermal archesporial cell is distinguishable, is *Tsuga*. Evidently *Larix* is meant here, but even this is not correct, for Saxton finds Strasburger's older statement to be unreliable.*

The book can hardly be regarded as an exhaustive treatment of the subject and no one could expect it within the scope of a single volume, but it is a clear and compact account of the whole group written in a somewhat conversational style. A factor contributing in no small measure to its clearness and readability is that with regard to controversial points the theory backed up by most evidence is given with only a passing reference to others.

The usefulness of the bibliography is slightly impaired by many inclusions of insignificant works and omission of some important and recent ones.

P. MAHESHWARI.

PALM, B. T. Ein neuer Embryosacktypus bei *Rudbeckia hirta* L. Bot. Notiser (Lund 1934, pp. 423-427).

Intensive research during the last 30 years has revealed several types of embryo-sacs and in a preliminary note Prof. Palm describes yet another type of embryo-sac development in *Rudbeckia hirta*, a member of the family Compositæ. In common with most of the Sympetalæ, the nucellus is extremely reduced and there is a single megaspore mother-cell. The development starts as in *Lilium*, no wall being laid down either after the homotypic or the heterotypic division, and the 4 megaspore nuclei are placed cross-wise. With the enlargement of the megaspore mother-cell, a vacuole appears in the centre. The nucleus at the upper end remains in its original position, while the other 3 nuclei pass down to the chalazal end of the cell. The micropylar megaspore nucleus now divides twice to produce 4 nuclei which form a normal egg apparatus and the upper polar nucleus. Of the three nuclei at the lower end, two (formed by the division of the lower dyad nucleus and lying closer to each other) remain undivided and give rise to two antipodal cells, while the third divides once to form a large antipodal cell and the lower polar nucleus. This is appreciably larger in size than the upper

* Another point that deserves mention is that the New Caledonian conifer *Acropyle* has still been retained in the Taxaceæ, though Sahni's work definitely indicates its relationships with the podocarps.

polar nucleus. The mature embryo-sac is thus 8-nucleate as in most other angiosperms, but the course of development differs from any that has been previously described.

The detailed paper of Prof. Palm will be awaited with interest. It serves to emphasize, however, that in all abnormal-looking embryo-sacs a very close series of stages should be sought for. It is perhaps due to inadequacy of material that RUTGERS made the mistake of reporting a 5-nucleate embryo-sac in *Moringa oleifera*, while actually it is 8-nucleate, as has been shown recently by PURI (Proc. Ind. Acad. Sci. Ser. B., 1934). A similar mistake seems to have been made by ARNOLDI, who reported a 4-nucleate embryo-sac in *Codium*, now shown to be 8-nucleate with ephemeral antipodals (LUNDBERG, Bot. Notiser, 1931). To take one more instance, even such a careful and experienced investigator as DAHLGREN reported a 4-nucleate embryo-sac in *Plumbago*, although it has now been shown (HAUPT, Bot. Gaz., 1934) to be a variation of the "Lilium-type", in which all eight nuclei are formed originally.

In the opinion of the reviewer, there are several plants like species of *Garcinia*, *Pandanus*, *Lemna*, *Cypripedium*, *Euphorbia*, *Casuarina*, *Loranthus*, and others, which deserve to be re-investigated. Material of all these genera is available in India and it is very probable that a detailed study of their embryo-sacs will reveal new facts, which the technique of earlier observers failed to disclose.

P. MAHESHWARI.



THERE are nearly 4,000 Institutions of pure and applied botany. There are between 60,000 and 70,000 botanists. There are about 1,000 periodicals concerned with botany! How can you keep in touch with all this activity? How can you find out what other botanists are doing and what new work they are planning. CHRONICA BOTANICA will help you. Subscribe to it and help with the compilation of the next volume.

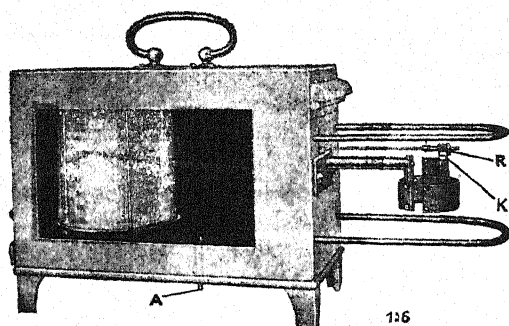
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Secretaries, who do not receive our preliminary circular, which will reach them annually before October 15th, are kindly requested to acquaint us of the fact at their earliest convenience, which will enable us to include them in our mailing list, and will ensure their receiving a copy of the questionnaire in December.

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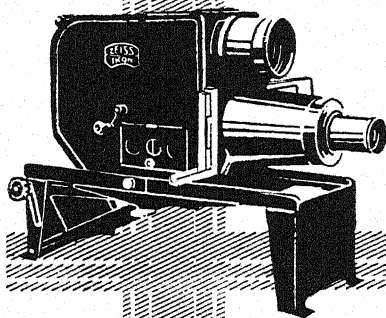
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VOL. XIV

JUNE 1935

No. 2

STUDIES IN THE DISEASES OF APPLES IN NORTHERN INDIA

I. A New Leaf-Spot Disease of Apples caused by *Oothecium indicum* n. sp.

BY

H. CHAUDHURI

AND

PUSHKAR NATH

(From the Department of Botany, Punjab University)

Received for publication on 1st December 1933

Introduction

In this series it is intended to study the different fungal diseases of the apple trees in Northern India, including the State of Kashmere. These are attacked by various fungal pests and many of these attack only the leaves. Most of the diseases of the leaves have a limited growth forming only isolated spots or holes. In certain cases the whole leaf may be killed or defoliation may occur. In the following pages a leaf-spot caused by a phomaceous fungus, viz., *Oothecium indicum* n. sp., has been described. It is quite common in Kashmere and the diseased material was collected from the Lal Mandi Garden at Srinagar (Kashmere).

Field Observations

It has been found to be more common in the orchards where the plants are crowded together. Isolated growing trees were seldom found to be affected. The spots first appear on the upper surface of the leaves as small scattered dots, and in the younger stages a white central region with a brownish margin may be seen. As they grow old, the spots develop a purplish rusty colour. Viewed from the undersurface the spots appear brownish. The number of spots in the leaves varies much. These are more or less circular in outline and have a definite margin; where two or more spots are situated near about, they may fuse and cover a large area and present a scorched appearance. Very minute black dotlike pycnidia are seen scattered over the spots. These pycnidia when crushed under a microscope, show small dark brown oval spores. The infected tissue becomes so very brittle that hand sections are very difficult to cut and microtome sections could only be cut after the tissue had been properly softened and infiltrated. In older spots the tissue crumbles down and holes are formed. Section of the diseased tissue showed the presence of hyphae.

Isolation of the Fungus

Small bits of infected tissue bearing pycnidia were placed in 0.1 per cent. mercuric chloride solution for 1-2 minutes and then washed in sterilised water several times. A bit of the diseased tissue was then crushed in a tube containing sterilised water. From this spore suspension, dilution culture on potato-glucose agar were made and incubated at 30°C. Young colonies were seen developing on the 5th day and isolations were made from single spore growths. Pycnidia appeared in 10-day old cultures. Examination of pycnidium showed the presence of spores like those found in the pycnidia on leaves in nature.

Inoculation Experiments

Inoculation experiments were performed in March, 1932 and cut leaves from a Lahore tree were used. Fresh leaves were washed well with 0.1 per cent. mercuric chloride solution and later rinsed several times in distilled water. These leaves were put in sterilised Petri dishes containing soaked cotton wool. Strong spore-suspension in distilled water was made by crushing a number of pycnidia from a culture tube. In the first set, inoculations were made by simply spraying the suspension over the surface of the leaf by means of a sterilised blower. In the second set a drop of suspension was placed over a marked area previously pricked with a sterilised needle. In the control sets only sterilised spore suspension was used instead of living spore suspension.

All these sets were placed in an incubator with a glass door at 25°C. Brownish spots appeared on the leaves of the second set on fourth day. In these prick inoculations were made. After 12 days minute dark brown pycnidia were seen on the leaf. Pycnidia developed in concentric manner. Examination of these pycnidia showed that they contained spores of the type found in nature. From the artificially inoculated leaves, the causal organisms were re-isolated.

The leaves in the first set where the spores were sprayed, did not show any sign of the disease. This shows that the germ tubes of the spores cannot enter through sound and uninjured tissue.

The leaves in the control sets remained healthy. It has been observed that in nature the disease enters through the punctures made by the sucking insects. Inoculations made on fruits, though repeated several times, failed to produce the disease.

Description and Identification of the Fungus

Pycnidia are abundantly formed. In nature pycnidia are minute while in artificially inoculated leaves pycnidia are quite big, fleshy, and glabrous, and are produced superficially on the surface of the leaf. This may be attributed to the favourable condition of moisture and temperature in which the inoculated leaves were placed. Ordinarily the pycnidia appear separately though sometimes two or three may be aggregated together. They vary in shape, being either globose, oval or more or less elongated. Their size may vary from $110-357.5 \mu \times 96.5-343.8 \mu$ on inoculated leaves. The spores are copiously formed inside the pycnidium and are either borne on very very short conidiophore or directly upon the inner wall of the pycnidium and the spores later lie free in the cavity of the pycnidium. The wall of the pycnidium is several layers thick and, when mature, breaks at some points, no ostiole being formed, and the spores escape in a mass. The spores are ordinarily elliptical or lemonshaped, though in some cases they may be slightly curved, unseptate, dark brown and smooth and usually about 1.5-2 times as long as they are broad. The average size of the spores, both in nature and in artificially inoculated leaves, is about the same, being $5.2 \mu \times 3.3 \mu$.

The absence of stroma and definite ostiole and the superficial nature of the pycnidia which are glabrous and usually lie separately, though sometimes crowded together and the shape of the spores, definitely shows it to be an *Oothecium*. As this species differs from the only other species, *O. megalosporium* Speg., in its size of pycnidia and spores and also in the number of spores and other characters, it is described as *Oothecium indicum* n. sp.

TABLE I showing the growth characters in various media.

Medium.	Maximum growth in 10 days. in mm.	Nature of colony and colour of substratum.	Aerial growth.	Mycelia width.	Clamp connections.	Pycnidia.	Size of Pycnidia.	Spores, their size.
Apple extract agar.	80 x 80	Compact, dark brown.	Normal dirty white.	Brown 1.9-3.7 μ	+	—	—	—
Asparagine starch agar.	50 x 47	Loose, hardly visible from substratum.	Absent.	Delicate hyaline 1.3-2.4 μ	—	—	—	—
Bean extract agar.	61 x 60	Loose, hardly visible from substratum.	Absent.	Delicate, hyaline 1.3-2.2 μ	—	—	—	—
Cook's medium.	71 x 70	Compact, brown.	Luxuriant, dense, fluffy white.	Light brown 2.4-6.2 μ	+	Abundant, scattered over medium.	137.5-288.8 x 110-233.8 μ Average 195.5 x 165 μ	Slightly covered some times 4.7-7.9 x 3.1-4 μ Average 5.6 x 3.3 μ .
Czapek's medium.	74 x 73	Compact, brown.	Luxuriant, dense, fluffy and white.	Hyaline or light brown 2.9-7.7 μ	+	Abundant, aggregated seldom scattered.	133.8-309.4 x 120-261.3 μ Average 206.3 x 168.2 μ .	4.2-6.6 x 2.8-3.5 μ Average 5.5 x 3.4 μ .
Prune extract agar.	72 x 72	Compact, dark brown.	Normal white, loose fluffy.	Light brownish, 2.4-7.1 μ	+	Abundant, scattered both towards centre and periphery.	137.5-453.8 x 137.5-440 μ Average 241.1 x 236 μ .	4.4-6.6 x 3.1-4.7 μ Average 5.2 x 3.9 μ

TABLE I showing the growth characters in various media—(continued).

Medium.	Maximum growth in 10 days. in mm.	Nature of colony and colour of substratum.	Aerial growth.	Mycelia Width.	Clamp connections.	Pycnidia.	Size of Pycnidia.	Spores, their size.
Potato glucose agar.	65 x 65	Compact brown.	Luxuriant white, dense, low & fluffy.	Light brown 2.8-5.5 μ	+	Abundant, scattered on agar and superficial.	110-316.3 x 96.2-243.5 μ Average 183.6 x 162.3 μ	4.4-7.1 x 2.8-4.1 μ Average 5.7 x 3.1 μ
Potato extract	53 x 52	Loose, white	Absent	Hyaline 2.4-4 μ	—	—	—	—
Robinson's medium	74 x 74	Loose, delicate, white	Absent	Hyaline 2.4-4.1 μ	—	—	—	—
Richards' medium	53 x 51	Delicate, loose, white	Absent	Hyaline 2.8-4.8 μ	—	—	—	—
Raulin's medium	74 x 73	Much compact, dark brown	Very luxuriant brown, fluffy	Young hyaline 2.5-3.7 μ old brown 3.1-7.1 μ	+	—	—	—
Maize starch	70 x 68	Compact brownish	Scanty	Light brown 2.5-5.6 μ	+	—	—	—
Plain agar	39 x 38	Loose, delicate, hardly visible	Absent	Hyaline 1.6-2.4 μ	—	—	—	—

Growth in Culture

This fungus grows readily in culture and its behaviour on various vegetative and synthetic media has been studied. The size of the hyphæ, length of the cells, and their contents vary a great deal in different culture media. It takes up a faint colouration in certain media, while in others, it may be almost dark. Young hyphæ which are rich with oily reserve vary in width from 1.9 to 7.7 μ . In many cultures, union between two neighbouring hyphæ or clamp-connection between two neighbouring cells may be seen. Table I shows the growth characters of the fungus in different media. Point inoculation in the centre of the Petri dish was made in all cases and the Petri dishes were incubated at 27°C. From Table I, the effect of media not only on the growth of the mycelium but also on pycnidia formation and sporulation will be clearly seen. Though a large number of Petri dishes were inoculated every time, it was found that no pycnidia formation took place in apple-extract agar, asparagin starch agar, bean-extract agar, potato extract agar, Robinson's in agar, Raulin's in agar, maize agar and in plain agar.

Effect of Concentration of Media on Growth

Next the effect of various concentrations of media on growth was tried. For this various concentrations of potato-glucose agar were tried. The plates were incubated at 28°C. The rate of spread of the fungus was found to increase with the increase in concentration of the media up to 8N and in higher concentrations, more compact growth took place. In 8N concentration the substratum presents a furrowed appearance. In lower concentrations zonal growth was very common and pycnidia appeared early.

Effect of Light and Darkness

Potato glucose agar plates were used for this. After point inoculation, one set was covered with black paper. These, along with a second set of plates which were not covered, were placed under strong electric light for 10 days in a dark room. It was found on examination that pycnidia formation occurred only in plates exposed to light and that the rate of growth was faster in darkness than in light.

Effect of Humidity

Cultures were grown in (1) saturated atmosphere, (2) moist atmosphere and (3) in dry atmosphere. It was noted that in the first two cases the rate of spread was similar though more fluffy growth took place in a saturated atmosphere and also there was no difference in pycnidial formation. But under dry conditions, the rate of growth was very slow, aerial hyphæ poorly developed and no pycnidia were formed.

Thermal Death Point.—By exposing spore suspensions for a period of 10 minutes to different temperatures and later plating these suspensions, it was found to be 52°C.

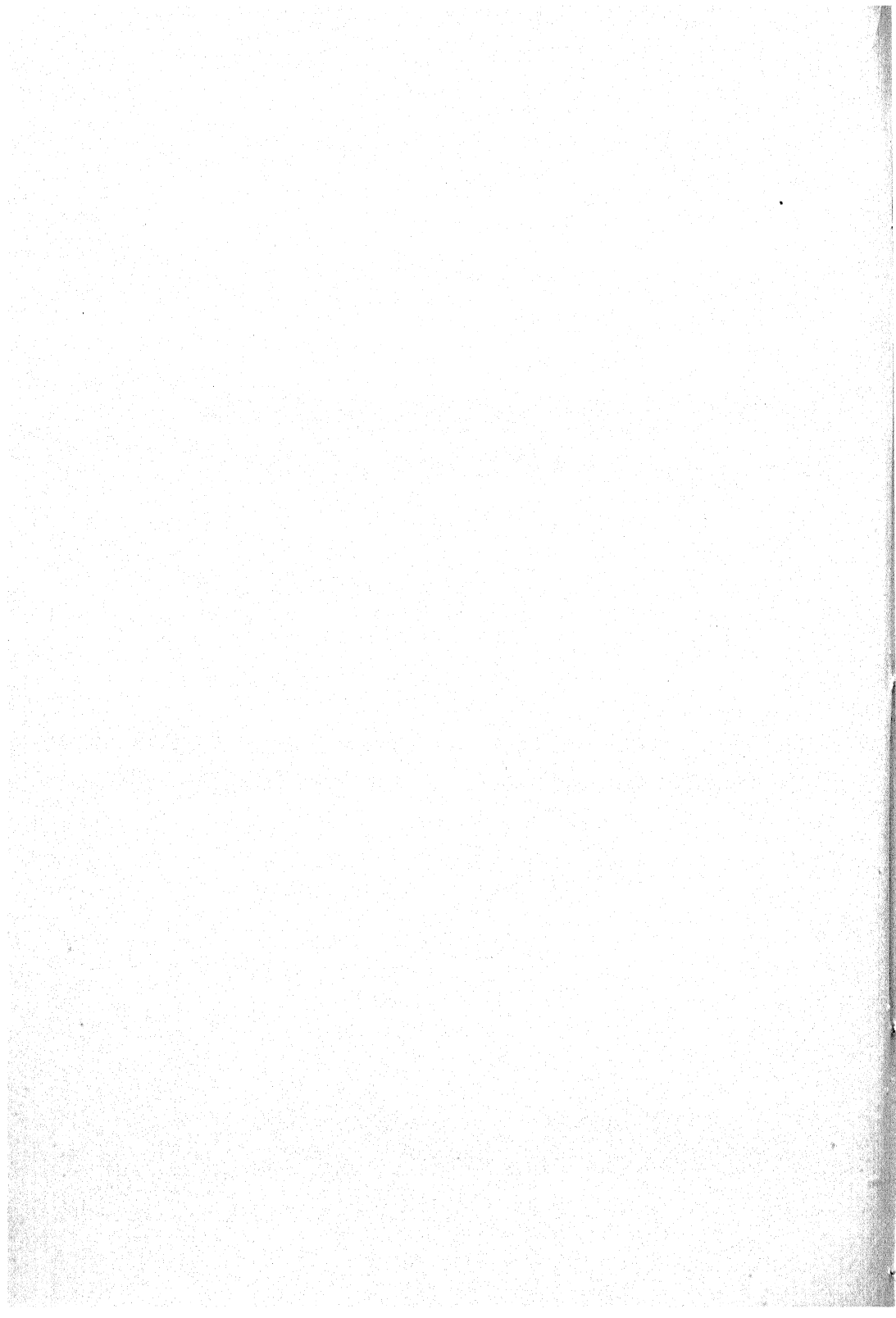
Summary

A new leaf disease of the apple plants caused by a phomaceous fungus has been described.

The casual organism has been isolated and described as *Oothecium indicum* n. sp.

By inoculation experiments, its pathogenicity has been established.

The growth characters of the fungus under different cultural conditions have been studied.



THE OSMOTIC AND SUCTION PRESSURES OF SOME SPECIES OF THE MANGROVE- VEGETATION

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Received for publication on 6th December 1933

The methods of determining the osmotic strength of the cell sap have been described and used by various authors like De Vries (1884), Pfeffer (1887), Stange (1892) and Janse (1887) but none of these workers studied the range of osmotic strength met with in plants growing in different localities.

Drabble and Lake (1905) made use of the method of plasmolysis of De Vries (1884) to determine the osmotic strength of the cell sap in plants growing under different environmental conditions. They found that xerophytes had in general higher osmotic strengths in the cells than mesophytes, but this characteristic does not help in retarding water loss but is for absorbing water through the leaves. Drabble and Drabble (1907) extended their observations to determine the relation between the osmotic strength of cell sap in plants and their physical environment. In their investigation these authors unlike others have given the osmotic strength in terms of millimeters of mercury. They determined the osmotic strength of forty-eight species growing in different localities like the bogs, gardens, woods, moorland, mountain brackish water and salt marshes and they concluded that the plants which had most difficulty in obtaining an adequate supply of water have a higher concentration of cell sap than those which were more favourably situated. The higher concentration of cell sap retards evaporation of water, depresses the freezing point of the sap and increases the absorption of water. Hill (1908) studied the osmotic properties of the root hairs of salt marsh plants and he confirmed the findings of Drabble and Drabble (1907). He found that the osmotic strength in different hairs of the same individual plant

varies, and it may vary in the root hairs of different individuals of the same species. The salt marsh plants have the power of adjusting their internal osmotic strength according to the salinity of their surroundings.

Sodium chloride is an important factor in the distribution of plants growing in salt marshes. It is found that a pure solution of sodium chloride has a toxic effect on all parts but when other salts are present along with it the toxicity disappears. In nature, however, there are a large number of plants which cannot grow in soil containing sodium chloride in small amounts, even when other salts are present, while some plants flourish in saline soils and these plants can be educated to endure certain salts. The latter fact was first pointed out by Stange (1892) in the case of root tips of the White Lupin and Scarlet Runner. Halket (1915) also found that *Salicornia ramosissima*, and *Suaeda maritima* Dum. could withstand the presence of 17 per cent of sodium chloride in the soil water. He also showed by a series of water culture experiments that species of *Salicornia* grew better in presence of sodium chloride than in its absence while *Suaeda maritima* Dum. was quite indifferent growth being equally vigorous with and without the salt. These differences between different species are due to the differences in permeability of the plasma membranes. The plasma membrane of salt plants are permeable to Sodium chloride like the leaves of ordinary plants which can also absorb salts as is shown by Boodle (1904) and others.

Plants living in salt marshes are exposed to different degrees of salinity in water at different times. The percentage of salts in soil water may vary from 0.74 to 2.77 as shown by Hill (1919). These variations in the salinity of water are shown by Osterhout (1906) to occur in the case of algæ attached to the sides of boats and steamers. As these plants live in places where the degree of salinity varies, it is expected that either the osmotic concentrations of the plant cells would correspondingly alter or absorb large amount of water both resulting in an injury to the plants. In the case of *Chaetomorpha*, Janse (1887) noticed a readjustment of osmotic pressure and in the case of *Codium tomentosum* Huds., Nathansohn (1901) found that there was a passage of chlorides from the plants to the surrounding liquid and *vice versa* until the concentration of the chloride became equal. Hill (1908) also showed that the root hairs of *Salicornia* can accommodate their internal osmotic pressure according to the changes in the salinity of the water surrounding them. It is an unsettled fact how the alteration in the osmotic pressure of the root is brought about. There are two possibilities either the plasma membrane is permeable to sodium chloride allowing its endosmosis or exosmosis or some chemical changes occur in the cells which bring about changes in the osmotic pressures.

The transpiration of the salt marsh plants is studied by Delf (1911, 1912) and Hill (1919) and they have found that the amount of water transpired from the halophytes varied but in all cases it was greater than that of a typical mesophytic plant and the maximum rate of transpiration was found in *Suaeda maritima* Dum. The results of Hill (1919) also show that the loss of water from succulent plants is considerably greater than that of the mesophytes. The high rate of transpiration of salt marsh plants is surprising and it means a greater amount of absorption of water. Hill (1919) has shown that aerial parts of plants can absorb water from the rain or dew and they also can absorb sea water when the plants get submerged at high tides on account of high concentrations of their cell sap. The same observations were made by Delf (1911) and Halket (1911) on *Salicornia*. It is also very likely that these plants can make use of the moisture in the air and living parts of the plant can draw water from the dead parts of the same individual.

The above review of the work done on the plants living in the salt marshes shows that the plants are exposed to different degrees of salinity at different times and as a result of these changes in the osmotic strength of the external medium the concentration of the cell sap of these plants varies accordingly. It is still an unsettled fact how the alteration of the osmotic strength of the cell sap of roots is brought about. It is either achieved by the changes in the permeability of the plasma membrane to the salts as their endosmosis or exosmosis occurs according to requirements or certain chemical changes occur in the cells as a result of a change in the osmotic concentration of the external medium and these chemical changes bring about changes in the osmotic pressure of the cell sap.

In order to add to the existing knowledge on the subject it is first necessary to determine the changes in the osmotic pressure of the external medium of the plants living in the salt marshes and to determine side by side the changes in the osmotic pressure of the cell sap of the roots of the plants growing there. More reliable data about the behaviour of these plants will be available by studying these changes under natural conditions than in artificial environments. The important point is the determination of the changes that occur in nature and how the plants are able to respond to these changes in the environment.

On the beaches and in the creeks in the Island of Bombay and Salsette are found salt marshes where mangrove vegetation occurs and the study of the osmotic pressure of the cell sap of the roots and of the surrounding media will be profitable and may throw some light on the problem discussed above.

It is now well established that the absorption of water depends upon the suction pressure of the roots and not upon their osmotic pressure so it is necessary to determine the suction force of the

roots and leaves of the plants growing in those localities. Such measurements of the suction pressures of these plants have not been done before.

The mangrove vegetation occurs at different places near the Bombay and Salsette islands. The following are the most important localities (1) Colaba Reclamation, South of Bombay, (2) Sewri, East of Bombay, (3) Thana, Ghodbunder and Bassein, North of Bombay, (4) Mahim and Bandra, North of Bombay.

As Colaba and Sewri are situated within easy distance of the Institute, samples of plants and soil water are collected from these places.

Three species from salt marsh vegetation, namely, (1) *Avicennia officinalis* Linn., (2) *Acanthus ilicifolius* Linn., (3) *Sonneratia apetala* Ham. are investigated.

The osmotic pressure of the sea water in the mangrove area is first determined. The samples of sea water were brought from Colaba Reclamation, South of Bombay, in December and January and the osmotic pressure determined by the indirect plasmolytic method and by the freezing point method. The following Table gives the results of these determinations. The osmotic pressure fluctuates between 24.8 and 25.0 atms.

It was also undertaken to study the osmotic pressure of the soil solution, as generally the roots absorb water in the soil and not from the upper surface. The soil solution was prepared according to the following method.

Method

Soil samples from the mangrove area were brought in paper bags and dried by keeping them spread thinly in paraffined glass dishes in places free from the laboratory fumes till the two consecutive weighings of the same sample were equal, then the soil was considered as completely dry.

The soil was gently powdered taking care that no stone was crushed. Fifty grams of the soil were mixed with 10 cc. of distilled water and allowed to remain in corked flasks for 24 hours. Then it was shaken vigorously and centrifuged for 25 to 30 minutes and the clear supernatant fluid was drawn off with a pipette. The osmotic pressure of the soil solution thus obtained was determined by the freezing point method, using the formula $(T - T^1) 12.05 =$ osmotic pressure in atmospheres, where T is the freezing point of distilled water T^1 the freezing point of soil solution and 12.05 the ratio of the osmotic pressure expressed in atmospheres to the freezing point depression in degrees centigrade of a solution of normal solute in water.

TABLE I

The osmotic pressure of sea water surrounding
the mangrove vegetation

Date	O. P. of Sea Water in atms.
15-12-1931	24·8
17-12-1931	25·0
19-12-1931	25·0
20-12-1931	25·0
22-12-1931	25·0
25-12-1931	24·8
31-12-1931	25·0
11- 1-1932	24·8

TABLE I-A

Osmotic pressure of soil solution and sea water by
freezing point method.

Date.	O. P. of soil solution in atms.	O. P. of sea water in atms.
9-5-1933	..	27·233
10-5-1933	20·485	..
11-5-1933	20·485	..
12-5-1933	20·485	26·269

The osmotic pressures of the stems, roots, leaves and pneumatophores of the mangrove plants are determined by the plasmolytic method. As the osmotic pressures are very high in these plants very strong solutions of sucrose and sodium chloride had to be used. The measurements of the osmotic pressure of the roots, stems and leaves are made from August to October. Table II gives the osmotic pressure of the roots, stems, leaves and pneumatophores of *Acanthus ilicifolius* L. The osmotic pressure of the plant rises from the roots towards the leaves. The osmotic pressure of the pneumatophores is nearly equal to that of the stem. The results show a rise in osmotic pressure from August to October. The

osmotic pressure of the roots rises from 56 atms. to 78.4 atms; of the stem from 60.0 atms. to 82.88 atms; of the pneumatophores from 41.32 atms. to 82.88 atms; and of the leaves from 64.9 atms. to 85.12 atms. The results show a considerably high osmotic pressure in these plants and it is lower during the monsoon and higher as the dry season approaches.

Table III gives the suction pressure of the roots, stems and leaves of *Avicennia officinalis* L. There is not much difference between the suction pressure and the osmotic pressure indicating that the wall pressure is not very great in these plants. The cells must not be in turgid condition and consequently the suction pressure is nearly as high as the osmotic pressure.

TABLE II

The osmotic pressure of the roots, stems and the leaves of *Acanthus ilicifolius* L.

Date.	O.P. of Roots in atms.	O.P. of stems in atms.	O.P. of leaves in atms.	O.P. of Pneumato- phore in atms.
2-8-1932	..	60.48	64.9	41.32
6-8-1932	56.0	67.2	..	53.76
14-8-1932	51.42	53.76
23-8-1932	51.42	67.44	71.68	62.76
24-8-1932	49.28	67.44	73.92	64.96
30-8-1932	49.28	71.92	76.16	44.8
3-9-1932	69.44	69.44	76.16	53.76
7-9-1932	69.44	76.16	76.16	53.76
12-9-1932	60.48	76.16	76.16	46.96
15-9-1932	64.96	75.04	80.64	56.0
19-9-1932	62.72	73.92	80.64	64.96
20-9-1932	64.96	78.4	80.64	73.92
28-9-1932	69.44	78.4	80.64	62.72
5-10-1932	78.4	82.88	85.12	82.88
11-5-1933	..	85.12	..	89.60

TABLE III

The suction pressure of the roots, stems and leaves
of *Avicennia officinalis* L.

Date.	S. P. of Root in atms.	S. P. of the Stem in atms.	S. P. of the leaves in atms.
11—7—1932	38·08	49·28	..
14—7—1932	40·3	49·28	..
15—7—1932	40·32	49·28	..
16—7—1932	40·32	49·28	..
19—7—1932	..	49·28	..
27—7—1932	..	49·28	56·58
30—7—1932	58·76
11—9—1932	..	67·2	76·16
24—9—1932	..	69·4	78·4
28—9—1932	..	78·4	80·64
5—10—1932	71·68	82·88	85·12

Table IV gives the osmotic pressure of the roots, stems and leaves of *Avicennia officinalis* L. The values of osmotic pressure are nearly as high as those obtained for the same organs of *Acanthus ilicifolius* L. The osmotic pressure in the root rises from 40 atms. to 73 atms; of the stem from 51 atms. to 85·12 atms. and leaf from 56 atms. to 87·6 atms.

The Table V gives the osmotic pressure of the stem and leaves of *Sonneratia apetala* Ham. The measurements of the osmotic pressure of the roots of this plant could not be made as the roots are very deep in the soil. The osmotic pressure of the stem rises from 60·48 atms. to 82·88 atms. and of the leaf from 62·72 atms. to 89·6 atms; from August to October Table VI gives the suction pressure of the stem and leaf of the same plant and there is no difference between the osmotic pressure and suction pressure in this plant.

TABLE IV

The osmotic pressure in the roots, stems and leaves
of *Avicennia officinalis* L.

Date.	O. P. of the Root in atms.	O. P. of the Stem in atms.	O. P. of the leaves in atms.
8-7-1932	40.3	51.5	56.0
11-7-1932	40.3	50.04	53.84
14-7-1932	42.56	50.04	..
15-7-1932	42.56	50.04	..
19-7-1932	..	51.52	56.00
27-7-1932	40.03	..	58.76
11-9-1932	53.7	69.44	78.88
24-9-1932	..	71.68	80.64
28-9-1932	67.2	80.64	82.64
5-10-1932	73.92	85.12	87.36

TABLE V

The osmotic pressure of the stems and leaves
of *Sonneratia apetala* Ham.

MATERIAL COLLECTED FROM SEWRI

Date.	O. P. of the stem in atms.	O. P. of the leaf in atms.
2-8-1932	60.48	62.72
9-8-1932	60.48	..
13-8-1932	..	69.44
15-8-1932	69.44	76.16
23-8-1932	60.48	73.92
29-8-1932	..	78.4
30-8-1932	64.96	73.92
6-9-1932	..	76.16
10-9-1932	..	85.12
14-9-1932	76.16	82.88
20-9-1932	73.92	76.16
23-9-1932	78.4	85.12
30-9-1932	85.12	87.36
4-10-1932	82.88	89.6

TABLE VI
The suction pressure in the stems and leaves of
Sonneratia apetala Ham.

Date.	S. P. of the stem in atms.	S. P. of the leaf in atms.
2-8 -1932	60.48	60.48
6-8 -1932	60.48	..
9-8 -1932	59.36	..
13-8 -1932	60.46	73.92
15-8 -1932	71.68	73.92
18-8 -1932	69.44	73.92
20-8 -1932	..	73.92
29-8 -1932	73.92	78.4
14-9 -1932	73.92	81.76
20-9 -1932	71.68	..
23-9 -1932	76.16	82.88
30-9 -1932	82.88	85.12
6-10-1932	82.64	87.36

Conclusion

The osmotic and suction pressures of the mangrove species rise from the roots towards the leaves as is the case in all plants. The osmotic pressure of these plants are very high as compared to other plants. The osmotic pressure of the sea water is nearly 25 atms. and, as the results show, the osmotic pressure of the sea water remains more or less uniform. As the osmotic pressures of the roots and leaves of these plants are much higher than those of the surrounding medium, the plants must not be experiencing great difficulty in the absorption of water.

The important feature of this investigation is the rise of osmotic pressure in these plants as the dry season sets in, though there is no increase in the osmotic pressure of the sea water. It is shown by Delf (1911), Halket (1911) and Hill (1919) that these halophytes transpire more vigorously than the mesophytes. During the monsoon months the water loss must not be great on account

of the very high humidity of the atmosphere during the rainy season. But as the humidity of the air becomes less the transpiratory activity becomes greater and the plant will lose more water. As the water loss increases the absorption increases and as the absorption increases, the quantity of salts absorbed also increases. Thus the concentration of the salts in the cell sap of these plants increases which produces higher osmotic pressures of these plants.

It was first put forward by Schimper (1903) that the mangrove plants avoid absorption of salts as salts are poisonous to them and therefore they possess xerophytic characteristics. It was then put forward that the above view of Schimper (1903) was untenable as the plants cannot avoid absorption of salts. Even though the absorption of water is lessened after a sufficient lapse of time the plants will have absorbed certain amounts of salts which will produce deleterious effects. According to the view of Warming (1914) the mangrove plants cannot absorb water as the osmotic pressure of water outside is very high and therefore they find it difficult to absorb water. As the plants are unable to absorb water, they reduce their water loss. The recent work quoted above on the contrary shows that the halophytes transpire more vigorously than the mesophytes indicating a fairly high absorption of water. The results obtained in this investigation show that the osmotic pressure of the roots is much greater than the osmotic pressure of sea water and therefore the absorption of water is not difficult as put forward by Warming (1914). The halophytes will lose more and more water as the humidity of the air decreases and the greater transpiratory activity favours the absorption of water.

The increase in osmotic pressure of the plants from July to October can be due to two reasons. Firstly the greater absorption of water may bring about concentration of salts which would result in an increase in the osmotic pressure. It means that the absorption of water from the soil is less than the water transpired. Secondly the increase in the osmotic pressure may be due to greater absorption of salts along with the greater absorption of water as the transpiratory activity increases. The plants cannot help absorbing salts along with the water. The increased absorption of salts brings about a greater accumulation of salts and consequently there is an increase in the osmotic pressure. The second reason given for the increase in the osmotic pressure seems to be more acceptable than the first. The mangrove plants have to pass through a period of dry season for eight months when the transpiratory activity is high. So it is not possible that the plants will do with less absorption of water as compared to the water loss which is very great according to the results of Delf (1911), Halket (1911) and Hill (1919). The plants on account of the high osmotic pressure will continue to absorb more water as water is lost. And the absorption of salt will follow the absorption of water. The greater concentration of

salts brought about will increase the osmotic pressure. The greater osmotic pressure will again increase the absorption of water. So the rise in osmotic pressure is not due to the rise in the osmotic pressure of the water in the soil as is supposed by many but is due to the greater absorption of salts along with the greater absorption of water when the transpiration is very vigorous.

During the monsoon the transpiratory activity will be lessened and the plants will absorb more water than is lost by transpiration and consequently the osmotic pressure of the plant organs will be lowered. The fall in the osmotic pressure will also be brought about on account of the distribution of salts over a large area on account of the production of new vegetative parts.

Summary

The relations between the osmotic strength of plants and their environment are studied since Drabble and Drabble (1907) first published their observations on the subject. The plants living in salt marshes are exposed to different degrees of salinity at different times and it is put forward that the concentration of the cell sap varies according to the changes in the external medium. It is an unsettled fact how the alteration of the osmotic strength of the roots is brought about. It is either achieved by changes in the permeability of the plasma membrane to the salts allowing their endosmosis or exosmosis or by chemical changes in cells which bring about changes in the osmotic pressures. It is therefore undertaken to study the suction pressures of the mangroves growing near Bombay at different times of the year to determine whether actual variations in the suction pressure occur or not. Such measurements of the suction pressure of the mangroves has not been done before. The plants investigated are *Acanthus ilicifolius* L., *Sonneratia apetala* Ham., and *Avicennia officinalis* L.

The suction pressure in each species is highest in leaves, medium in stems and lowest in roots. There is a marked increase in the suction pressure in each species from August to October. It is shown by Delf. (1911), Halket (1911) and Hill (1919) that halophytes transpire more vigorously than mesophytes. The halophytes will naturally lose more water in the dry season than during the monsoon when the air is very humid and the higher suction pressure during the dry months may be responsible for the greater absorption of water. It is possible that the rise in the suction pressure is brought about by the vigorous transpiration which may bring about greater absorption of salts along with water into the cells rather than by any one of the two possibilities indicated above.

The authors record here their deep sense of gratitude to Professor R. H. Dastur, Head of the Botany Department, Royal Institute of Science, Bombay, for his able guidance and for offering them all the facilities that he could command.

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STUDIES IN THE DISEASES OF APPLES IN NORTHERN INDIA

II. A Short Note on Apple Scab due to *Fusicladium dendriticum* Fuckel

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Received for publication on 10th December 1933

Introduction

Apple scab is one of the most widely distributed diseases of the apple. It is caused by *Fusicladium dendriticum* F. which has long been shown to be conidial stage of the fungus *Venturia inæqualis* Cook. Upon the fruit it forms black sooty spots which greatly lower the market value of the fruit. Large number of these spotted apples was collected from Kashmir during August-September 1930 and brought down to Lahore. Only a few specimens of leaves could be collected after a thorough search as the affected leaves readily fall down. Later on the same fungus was seen at Lahore in early spring. Here the leaves were found to be attacked severely. It occurred commonly on both young and old trees and nearly all the plants of that area were affected. When a branch is touched these diseased leaves fall off.

The Fungus in Nature

The fungus attacks leaves, twigs, blossom and the fruits, though it is seen commonly on leaves and fruits.

The Leaf:—The infection on leaves is of common occurrence at Lahore. The first noticeable sign is a light olivaceous discolouration but in most cases the old spots present a dark sooty appearance. The spots vary in number; they may be from one to twenty or more on a single leaf. When a large number of spots occur, they are closely situated as a result of which considerable curling may take place in many leaves. The tissue beneath the spots may later turn brown and become a dead patch. It has been noticed that the lower side of the leaf is commonly spotted but the upper side may be equally affected.

On the Fruit

Upon the fruit also dark sooty spots with a definite outline are formed. As the fruits mature the spots enlarge. A typical spot has a dark brown corky portion in the middle with a black periphery. In most cases the cuticle around the spot becomes more or less loosened and forms a white papery margin round the entire spot which is distinctly marked off from the black area lying underneath. Very old scab spots present a corky look. A large number of these spots may be present on the fruit. In such cases neighbouring spots may fuse forming an elongated large spot. In extreme cases when the fruits are severely attacked it may crack. It has been noticed that a large variety of apples is affected and the writer collected six varieties which were thus spotted.

Morphology of the Fungus

In order to study the morphology of the fungus, hand and microtomic sections 8 and 10 μ thick were cut. The material was fixed in chrome-acetic acid for 24 hours, washed in slow moving water for 24 hours, the succeeding processes being the same as used for paraffin sections. The sections were stained with Delafield's hæmatoxylin and eosin. In hand sections, gentian violet also gave good results. As the conidiophores were brown, hand sections were not usually stained. Microscopic examination of these slides showed that the mycelial layer of the fungus was situated below the epidermis. This layer in the case of fruit is quite broad. In many cases the epidermis with the cuticle is thrown off and the mycelial layer is exposed. The hyphæ form a compact pseudoparenchymatous layer. In the leaves the mycelial layer is a narrow one and cannot always be marked off distinctly. In the case of fruit the cells below the mycelial layer become much thickened and corky, and the cell cavities are much reduced. The corky cells are a few layers deep.

The sooty growth on the leaves and fruits consists mainly of dark coloured conidiophores which are dense and grow erect on the surface. These arise from the subepidermal layer of mycelia. They break off the epidermis and cuticle and become exposed as sooty patches over the surface. The colour of the conidiophores is dark brown in the case of fruit and light brown in case of leaves. On the fruit they are erect, unbranched, smooth walled and in some cases septate. They are about four to six times as long as they are broad. Their breadth varies from 4.4 to 6.2 μ (average 5.2 μ .) The conidia are borne singly and terminally on the conidiophores. They are brownish in colour and variable in size and are attached by their broad end to the conidiophore. They are unseptate, obclavate or lanceolate with more or less an acute apex and a broad base. They vary from 15.8 to 23.7 \times 7.1 to 11 μ .

Germination of Spores

Though a very large number of media was tried, the conidiospores from the Kashmere Apples, which were nearly three months old and were kept in a refrigerator at 3°C., could never be germinated. It was considered that this long exposure to low temperature had caused the viability to be lost. Later in January following, a few scabbed apples which were never put in cold storage, were obtained, but the conidiospores from these also failed to germinate in any media. Hanging drop cultures in apple extracts, glucose solution (1 per cent.) and in water at various temperatures between 10 and 30°C. were of no use.

Later again in the month of April 1932, fresh material of scabbed leaves was collected from Lahore. Two sets of hanging drop cultures were arranged in the usual way in 1 per cent glucose solution and water. One set was incubated at 30°C. and the other kept in a cool incubator at a temperature of 10-12°C. Observations after short intervals showed that conidia germinated after ten to twelve hours in the second set kept at 10-12°C. In the other set incubated at 30°C. no germination took place even though kept for more than three days. Before germination each spore swells up and may elongate considerably. The exosporium usually bursts at one point and the endosporium protrudes forward forming a short germ tube. The germ tube usually arises from the pointed end or it may arise from the broad end of the spore. When the spore becomes septate the germ tube arises from one of the cells along side wall but sometimes two germ tubes are given out from the two cells. The germ tube is hyaline, thin and it usually swells up near the tip forming an appressorium, but the formation of appressoria is not universal.

From the above it is evident that only fresh spores are capable of germination and that only when the temperature remains low. Conidia lose the power of germination after a short period. Hence the conidia of previous season are incapable of bringing about infection the following season.

Summary

Apple scab due to *Fusicladium dendriticum* F. has been noticed in North India and Kashmere and described.

Only the conidial stage of the fungus has been found on leaves and fruits commonly.

The conidia has been found to lose the power of germination after some time and consequently cannot bring about infection the following year. Fresh conidia germinate readily in presence of moisture at a temperature between 10-12°C.

The germ-tube is often swollen at the tip which acts as a sort of appressorium.

ON THE CYTOLOGY OF *PENNISETUM TYPHOIDEUM* RICH

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Received for publication on 29th November 1933

The chromosomes of *Pennisetum typhoideum* were reported by Rao (1929) to be extremely large in size when compared with those of many other crop plants of Southern India. On this account the writer thought a study of the cytology of this plant would yield some interesting observations, and, with this aim in view, a sample of bulk seed was obtained from the Millet Specialist, Coimbatore, to whom thanks are due. Though some plants were raised from Coimbatore seeds the material studied was from stray plants of *Pennisetum*, growing in the fields round about Bangalore. Incidentally the morphology of the anther and ovary is also included within the scope of this study.

Material and Methods

The flowers of *Pennisetum* appear in a thick conical terminal cluster. When they are exposed at the top of the plant their essential organs will have undergone the necessary meiotic divisions. Earlier stages could be got only when the terminal cluster is still hidden within the leaves. Various developmental stages were collected and fixed right away in the field between 12 and 2 p.m. in a solution of Allen's modified Bouin, with a previous dipping in acetic alcohol. The material after being washed, was run through various grades of alcohol and xylol and imbedded in paraffin. The flowers of *Pennisetum* possess two glumes and a palea, without any lodicules. The palea and the glumes in the very early stages, do not offer any resistance to being cut, but later when they get lignified, afford immense trouble. The very young flowers were run as such, but the older ones were deprived of their coverings in 30 per cent. alcohol, and only the stamens with the gynœcium were prepared for dehydration and embedding. Though this process involved some patient and tedious work the results obtained amply justified the trouble and time taken to remove the glumes from the flowers. Sections of varying thickness from 8 μ to 15 μ were cut to suit the preparation of anther and ovary and an attempt was made to study the development of the anther and ovary including the earlier embryology.

Study of Anther

The anther could be followed from the earliest stage of its development. The archesporial plate which consists of 4 to 6 cells in its length and 2 to 3 cells in its breadth, cuts off an outer parietal layer, (fig. 1) before it starts to function as the sporogenous tissue. This outer layer divides once to form two parietal layers (figs. 2 and 3) which protect the sporogenous mass. Later, the inner parietal layer divides once more to form the inner tapetum and the outer wall layer (fig. 4). At this time and in future the anther has the epidermis, two wall layers, tapetum and the inner sporogenous mass. The tapetal cells very soon enlarge in size being filled in by a highly granular protoplasmic mass (fig. 5). The peculiarity of the tapetal cells is that they are always uninucleate. The sporogenous mass, having undergone several divisions, is now prepared for reduction division.

Meiosis

The prophasic changes commencing from leptanema could be easily followed. The thin strands of the very early prophase soon approximate to fuse with their homologous partners and the threads get very thick and conspicuous by this time. Very soon the contraction starts and results in a thick knot of chromatin, which is shoved away towards one side of the nuclear membrane. This phenomenon has no significance according to Darlington (1932), who considers this to be an artefact, such an occurrence not having been found in living material. The contracted knot soon opens out to form free radiating loops which fill the cavity of the nucleus. The chromatin threads powerfully contract and the diplotanema shows thick threads forming chiasmata. The chiasmata from the early diplotanema could not be traced as the threads are extremely long and almost intertwined without any trace of individuality. The late diplotanema (fig. 6) shows clearly three chiasmata for each bivalent, two of them terminal and one interstitial. The noteworthy feature is the uniformity in the distribution of chiasmata in all the bivalents. The early diakinesis (fig. 7) represents seven ring shaped bivalents, two of them showing the profile. The terminalisation of the interstitial chiasma of the late diplotanema seems to be mostly effected by the repulsive force which starts between the two pairs of chromatids. The chiasmata are completely terminalised in all the bivalents. The tension with which the two pairs of chromatids are pulled joined by chiasmata only at the ends is a great proof of the immense repulsive force which exists between the halves of the bivalent and, as Darlington (1932) says, the repulsive force increases with the coming separation of the two halves of the bivalent at the anaphase. Open rings reported in *Oenothera missouriensis* by Hedayatullah (1933) in support of an end to end fusion of pairs of chromatids, could not be traced in this material. In some cases

the arms of the ring were found to be a bit apart, but even then they were always seen to be united by pairs of fine strands from their ends. Another interesting feature, as very often observed in diakinesis, is that the bivalents seem to be grouped four at one pole and three at another pole (fig. 8) not exactly opposite. On emerging out in this manner they are scattered in the nuclear cavity to be later attached by the spindle fibres which arise at different points outside the nuclear cavity, to group subsequently into two poles with the disappearance of the nuclear membrane. The early anaphase is marked by the heart shaped paired chromatids, all of them having a median spindle attachment (fig. 9), the heart shape having resulted from further contraction in the length of the ringed bivalent and an extension on the top due to the pull of spindle fibres. As the individuals are pulled apart the terminal chiasmata are forced out one after the other. The closeness of the contact between the paired chromatids in the bivalent by the formation of chiasmata is noticed by the fine paired strands which extend from one pair to the other at early anaphase even after the bodies of the chromatid pairs are separated.

For purposes of comparison, the prophasic changes as observed in the meiosis of the megaspore have been shown in figures 10 to 13. The early diakinesis has five ringed bivalents and two more still having an interstitial chiasma each. Metaphase configuration (fig. 11) is just the same as that of the microspore having 7 ringed bivalents with median spindle attachment (fig. 12). The separation of the bivalent halves also (fig. 12) is just in the same manner, the volume of chromatin remaining almost the same as that of the microspore meiosis.

The anaphase chromosomes as they reach the poles begin to show a median faint line (fig. 14) along which separation of chromosomes takes place in the succeeding division. The nuclear membrane is formed, the nucleolus reappears followed by the formation of an evanescent cell plate in the late telophase. The chromosome halves begin to repel towards the end and seem to be retained intact on account of their median contact, very often showing configurations of X (fig. 15). The median spindle fibres begin to wane, while the distal ones remain and become more pronounced in the second division. Very soon the second division follows (fig. 16) the chromosomes in the metaphase assuming the form of X described by Darlington (1932) as two chromatids held at the attachment constriction.

Lagging chromosomes are not an uncommon feature in the meiotic division. A case of a laggard along with the full complement of chromosomes is represented in figure 17. The lagging nature seems to be due to some trouble in the terminalisation of chiasmata. The laggard clearly shows two free ends on one side

and a continuous arm on the other side, which has resulted, due to some interlocking in the pull.

A mitotic figure (fig. 18) was easily obtained from the mass of sporogenous cells undergoing division to form pollen mother cells. The diploid number of fourteen chromosomes could be well seen in the metaphase. All the chromosomes are of the same length and breadth showing neither trabants nor constrictions. This result seems to be slightly different from the observation of Rao (1929) who has seen chromosomes of different shapes and sizes, hence of separate homology, in the mitosis of root tips of the same material.

Pollen Formation

The formation of the pollen grain is isobilateral. The pollen grain even in old anthers (fig. 26) shows only a single nucleus, its division being postponed until the formation of the pollen tube. A very prominent weak spot is noticed in every pollen grain. This serves as the pore through which the pollen tube emerges on germination.

Development of the Ovary and the Ovule

Very young flowers easily indicate the origin of the carpels which are always two in number (fig. 19), one thicker than the other. The two carpels have separate branches of vascular traces which emerge from a common supply. The thickness of one of the carpels is due to its bearing the primordium for the ovule, in which the hypodermal archesporial cell is well marked. While the carpel bearing the ovule on one side grows in its girth the other grows in its length and soon overtakes the former (fig. 20) to form the style and stigma on the top. The ovule as seen at this stage is laterally orthotropous having only the inner integument coming up half way. The archesporium is directly transformed into the megaspore mother cell (fig. 21) or in some cases a parietal cell is also cut off before the megaspore mother cell is established. The megaspore mother cell after its division forms two cells (fig. 22) which in their turn divide to form the linear tetrad (fig. 23). This kind of development is quite in correspondence with the formation of megaspores in *Poa pratensis* (Anderson 1927). The upper three megaspores disorganise while the lowermost enlarges to form the embryo-sac. The changes in the embryo-sac nucleus follow the usual course in ultimately forming eight nuclei out of which two from the poles form the fusion nucleus. Figure 24 has been reconstructed from two serial sections to bring out the full contents of the embryo-sac, the noteworthy feature here being the prominent chromatin loops in all the nuclei both at the antipodal and chalazal ends. In *Poa pratensis* also an eight nucleate sac is established but the peculiarity there is that two or three embryo-sacs fuse together in the same nucellus very often giving rise to polyembryony. The ovule in the meanwhile, on account of its lop-sided development, undergoes a twist

to turn towards the base of the ovary and becomes anatropous (fig. 25) being covered completely by the inner integument. The outer integument on the top stops growth half way while on the lower side it forms a thick pad through which the pollen tube has got to make its way before it enters the micropyle. Although a severe search was made, stages in fertilisation could not be secured though post-fertilisation stages were abundant. With the fertilisation of the egg the endosperm nucleus also divides rapidly by free nuclear division and the resulting nuclei distribute themselves freely along the periphery of the cavity of embryo-sac (fig. 27) joined together by thick cytoplasmic strands. A significant feature of the ovule at this time is that it shows a few cells* below the embryo-sac rendered very prominent by their large size and high protoplasmic content. These cells probably act as nourishing cells, disorganising only with the growth of the embryo (antipodal haustorium).

Embryo

The pro-embryo is in accordance with many of the monocots, having only three cells (fig. 28), a basal cell and two terminal cells. The first to divide vertically is the topmost (fig. 29), the next one following suit very soon (fig. 30). A similar condition of a three celled pro-embryo whose top cell after undergoing divisions forms the scutellum and upper edge of coleoptile, the middle cell forms the hypocotyl, stem tip and root point, and whose lower cell forms the epiblast, coleorrhiza and root cap, is described by Soueges (1924) for *Poa annua*. Later stages in embryo formation could not be obtained as even by this time the material gets hard by the formation of cutin, pectin and lignin, thus rendering the penetration of paraffin in xylol impossible. The method employed by Anderson in cutting the ovaries by a freezing microtome or by directly embedding ovaries in paraffin and cutting seems to be a favourable one for the study of the old embryo.

One thing remarkable about the ovule is that even the unfertilised ones keep pace with the fertilised ones upto a certain limit to get disorganised and shrivelled up only very late, so that the writer found to his disappointment that many of the large sized ovaries he so carefully teased out from the glumes expecting them to show some nice embryo, proved to be the unfertile ones.

Summary

(1) Archegonium of the microsporangium is a plate of four to six by two to three cells. The first division separates the parietal from the sporangial. The parietal forms the tapetum and two wall layers.

*It was later found that these are haustorial cells developed from the antipodal cells within the embryo sac, and later assume such enormous proportions as to appear outside the embryo sac.

(2) Stages in meiosis of microspore have been closely followed. Late diplonema shows three chiasmata, two terminal and one interstitial for all the bivalents. At diakinesis the interstitial chiasma also gets terminalised giving the appearance of a ring to the bivalents. Seven rings are clearly seen. The split for second division is observed at early telophase of the first division.

(3) Meiotic figures in megaspore mother cell also have been observed in detail and these correspond in every respect with those of microspore.

(4) The carpels have a distinct double origin. The formation of the embryo-sac and its contents is quite normal. A peculiar feeding tissue found below the embryo-sac has been described.

(5) Pro-embryo consists of three cells. The basal cell remaining as such, the upper two divide early.

My grateful thanks are due to Dr. M. A. Sampathkumaran under whose personal care and guidance this work was done.

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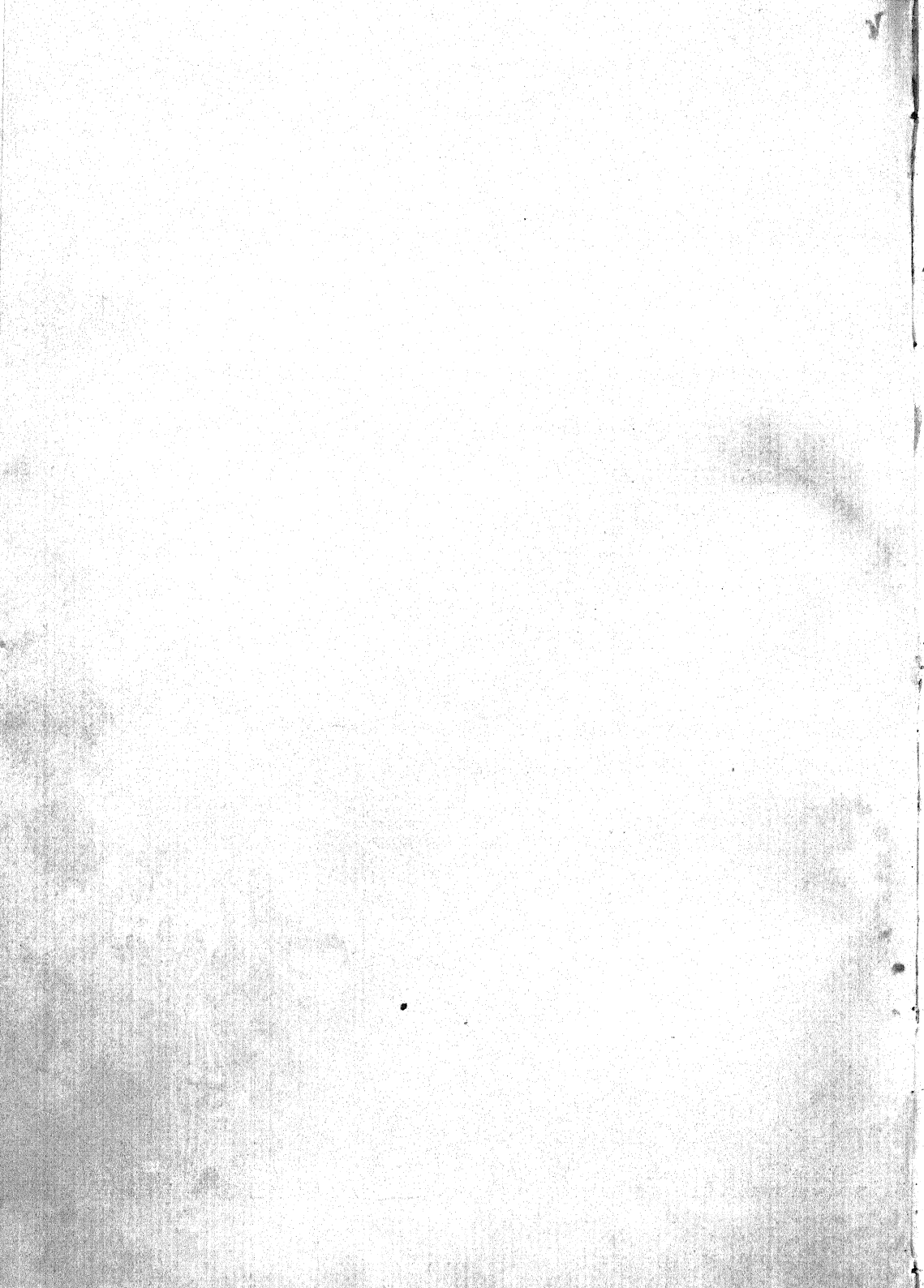
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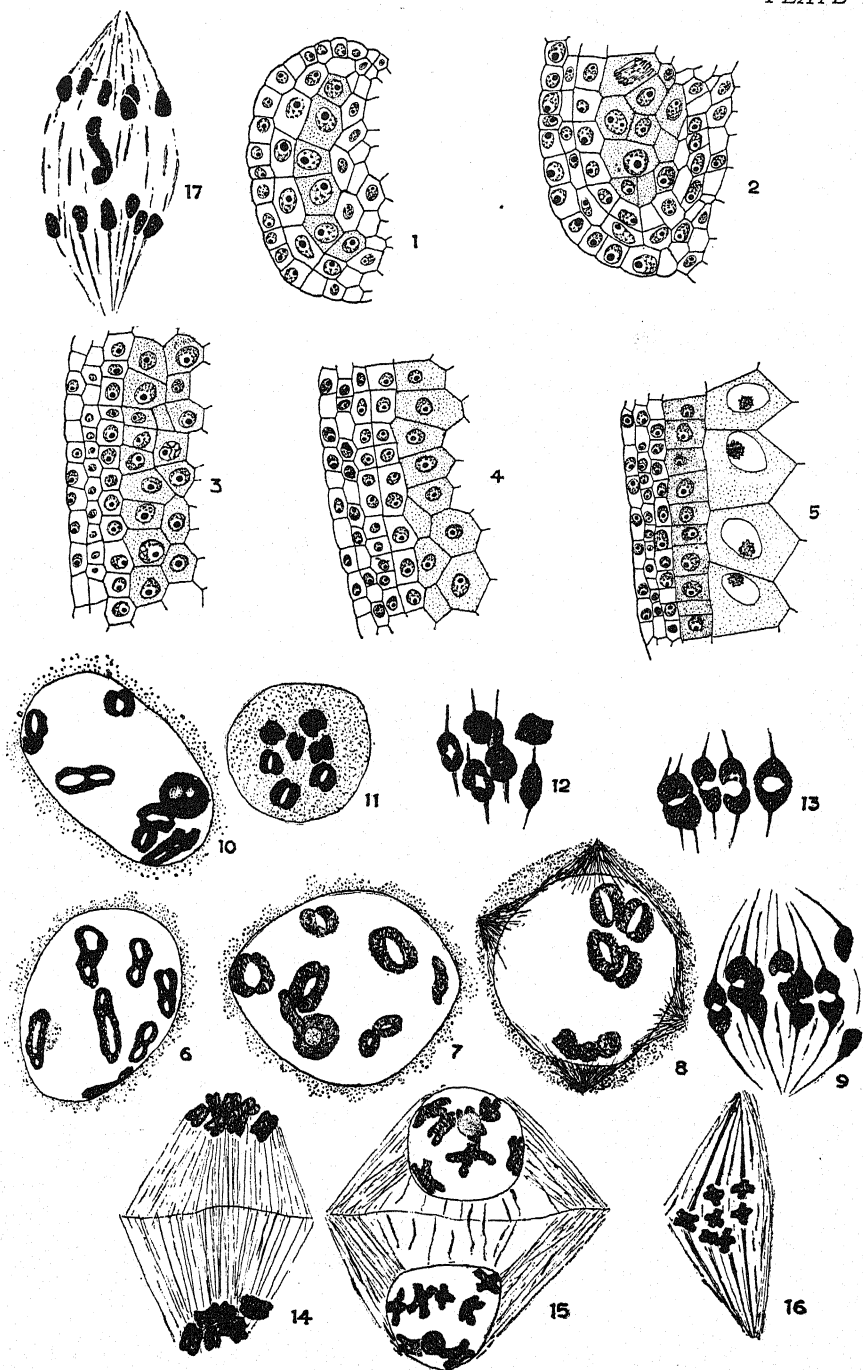
The nuclear figures were drawn with the aid of a Zeichen Apparat employing a Zeiss oil immersion N. A. 1.3×90 . The numerical figures against the explanations of figures indicate the original magnification to which the figures were drawn.

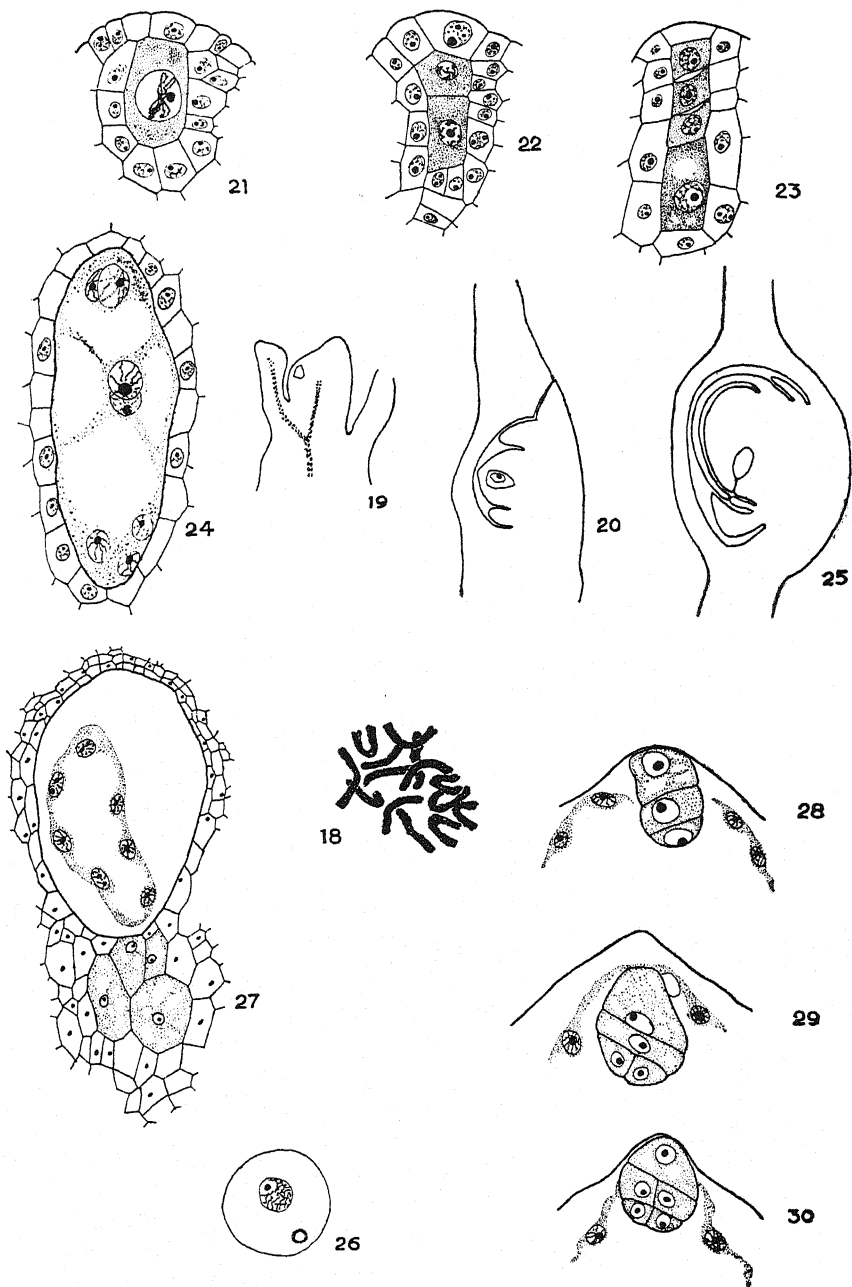
Explanation of Figures

- Fig. 1. Section of young anther to show the first division in archesporium separating the parietal layer.
- Figs. 2 and 3. Division of parietal into two layers, early and late.
- Fig. 4. Division of inner into the wall layer and tapetum.
- Fig. 5. Well organised tapetum, wall layers, and p.m.c.

- Fig. 6. p.m.c. nucleus in late diplotene.
- Fig. 7. Same in early diakinesis.
- Fig. 8. Same in late diakinesis.
- Fig. 9. Same in early anaphase.
- Fig. 10. Megaspore mother nucleus in late diplotene.
- Figs. 11 and 12. Same in metaphase.
- Fig. 13. Same in early anaphase.
- Fig. 14. p.m.c. nucleus in early telophase.
- Fig. 15. Same in late telophase.
- Fig. 16. Metaphase in second division.
- Fig. 17. Anaphase in first division with a laggard
- Fig. 18. Mitotic chromosomes from archesporium.
- Fig. 19. Diagram to show the origin of two carpels in young flower.
- Fig. 20. Diagram showing the fusion of two carpels with the formation of the lateral ovule with inner integument.
- Fig. 21. Hypodermal megaspore mother cell.
- Fig. 22. First division in the same.
- Fig. 23. Linear tetrad of megaspores.
- Fig. 24. Embryo-sac with full complement (a reconstruction from two sections).
- Fig. 25. Diagram of mature ovary showing the bent ovule with the integuments.
- Fig. 26. Microspore from a mature anther.
- Fig. 27. Nourishing cells below embryo-sac.
- Fig. 28. Pro-embryo of three cells.
- Fig. 29. Pro-embryo with vertical division in top cell.









STUDIES ON THE FEMALE GAMETOPHYTE IN SOLANACEÆ

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Received for publication on 11th November 1933

The importance of the study of the embryology of plants in the elucidation of the phylogenetic relationships between different members of plant kingdom has been realised in recent years. Solanaceæ, as is well known, contains more than seventeen hundred species some of which are of great economic importance. The literature on the subject, specially regarding the female gametophyte, is, however, very meagre. The first work in this direction was due to Guignard (9) who observed a normal type of embryo-sac development in *Cestrum splendens* and in *Nicotiana Tabacum*. Soueges (16), Palm (13) and Svensson (17) working respectively with *Atropa Belladonna*, *Nicotiana 'Delitabac'* and *Hyoscyamus niger* came to the same conclusion as Guignard. Soueges (16), however, has not studied the development of the female gametophyte thoroughly. Nanetti (12) in *Solanum muricatum*, and Young (19) in *Solanum tuberosum* on the contrary found a "Lilium type" of development of the embryo-sac. Cooper (7) very recently has also found a "normal type" of development of the female gametophyte in *Lycopersicum esculentum*. From the previous work done by the author (3) in *Solanum Melongena* it was thought that a comprehensive account of the development of the female gametophyte in Solanaceæ will be of great value in determining the systematic and phylogenetic relationships of the different species and might incidentally settle the controversy regarding the type of development of the female gametophyte in this family.

Material and Methods

The material for the present investigation was obtained from the following species :—

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| 1. <i>Solanum nigrum</i> L. | 2. <i>Lycopersicum esculentum</i> Mill. |
| 3. <i>Physalis minima</i> L. | 4. <i>Physalis peruviana</i> L. |
| 5. <i>Withania somnifera</i> Dum. | 6. <i>Datura fastuosa</i> L. |
| 7. <i>Cestrum diurnum</i> L. | 8. <i>Cestrum nocturnum</i> L. |
| 9. <i>Nicotiana plumbaginifolia</i> Viv. | 10. <i>Petunia nyctaginiflora</i> Juss. |
| 11. <i>Salpiglossis sinuata</i> Ruiz. | 12. <i>Brunfelsia americana</i> Sw. |

They were collected from the University College compound, Royal Botanical Gardens, Sibpur, and other places round about Calcutta. Ovaries were dissected out from flower buds on bright days and fixed immediately in a number of fixatives. Allen's modified Bouin's fluid and Licent's fluid gave best results. After fixation the material was washed and then dehydrated in the usual way. It was cleared in cedar wood oil and occasionally with xylol. The material was embedded in paraffin and sections were cut 8 to 14 μ thick, according to the nature of the study. Heidenhain's iron alum hæmatoxylin with or without a counter stain was chiefly used. "Orange G" and "Light Green" were mainly used as counter stains.

Development of Ovules

The sequence of development of the ovule is very uniform throughout this family. The placenta is very thick and massive, and in earlier stages of development consists of a homogeneous mass of parenchymatous tissue. The first indication of the development of the ovules is noted in the epidermis and three or four layers of sub-epidermal cells, which are rich in cytoplasm and contain conspicuous nuclei. Groups of cells, "ovule initials" differentiate out in large numbers from this sub-epidermal tissue and are distinguished from the rest of the cells by their marked activity. They divide first more actively in one direction and the tissue thus produced begins to elongate. To keep pace with the activity of the sub-epidermal tissue the epidermal cells also divide repeatedly anticlinally. The epidermis of the placenta loses its evenness at this stage and the identity of the numerous ovules becomes apparent. (Fig. 135.) The ovules at first appear as blunt papillate processes, but soon become slightly bent with the tips directed laterally, due to pronounced unilateral growth. The archesporial cell differentiates out at this stage.

Origin and Development of the Integument

The integumental tissue develops after the archesporial cell is well differentiated. It first appears as an annular outgrowth from the base of the nucellus and in longitudinal section appears as two notches. Before the heterotypic division is completed, the integument almost overtops the nucellus. In *Physalis peruviana* it has been observed that the nucellus lies completely imbedded in the integumental tissue before the megaspore mother cell completes the heterotypic division (fig. 32). Due to unequal growth of the integument the ovule gradually curves towards the base. The limit of this curvature varies in different species, giving different forms to the ovules. In *Cestrum* for example, the ovules are perfectly campylotropous, whereas in *Nicotiana* and in *Petunia* they are anatropous. In the other genera studied the ovules are half anatropous. Chatin (6) also found anatropous ovules in *Nicotiana*, and according to Cooper (7) the ovules are also anatropous in *Lycopersicum*. The funiculus is very short and stumpy in all the species except in *Petunia nyctaginiflora* and in *Nicotiana plumbaginifolia*, where it is comparatively slender.

In all the species investigated the integument in the earlier stages of its development consists of three to four cell layers, but becomes massive when mature. It is also uniformly thick in all the species except in species of *Cestrum*, and in *Physalis peruviana*, where it attains larger dimensions. In the former genus the cells are also bigger (figs. 61 and 62). According to Soueges (16) the fully mature integument shows a high degree of histological differentiation of its tissues. He has distinguished the fully mature integument into three layers, as follows:—

- (1) "Assise externe"—or the single layer of epidermal cells.
- (2) "Assise interne"—or "assise digestive"—the innermost layer of cells covering the embryo-sac.

- (3) "Partie moyene"—the intermediate layers of cells which again consists of two zones—"Zone externe" and "Zone interne."

On the histological differentiation of these three layers he has been able to classify the principal genera under Solanaceæ.

The nucellus proper does not divide and is composed of a single layer of cells. With the development of the embryosac the nucellus begins to degenerate and in fully mature embryosac no trace of it is noted. The mature embryo-sac lies buried in the massive integumental tissue leaving only a narrow passage leading to the micropylar end of the embryo-sac.

The Archegonium

The archegonial cell as a rule differentiates out in hypodermis, just under the bent tip of the ovule. In all the species investigated this differentiation takes place before the differentiation of the integument. The archegonial cell is comparatively bigger than the surrounding cells and contains a conspicuous nucleus and dense cytoplasm. The presence of more than one archegonial cell is very common in most of the species investigated. Two archegonial cells lying side by side, or one below the other, or even a group of four cells, have been observed in some of the species investigated (figs. 8, 9, 10, 33, 34, 76, 77, 100, 101, 102, 114 and 115). In the light of the present investigation it appears that the origin of these multiple archegonia is mainly due to the subsequent transverse, or longitudinal division of the initial hypodermal archegonial cell. As in some preparations two archegonial cells lying one below the other and without any partition wall between them, or the transverse division of one of the two archegonial cells lying side by side have been observed (Text figs. 33, 34, 35, 100 and 113). The division of the archegonial cell into a "Cover cell" however, has not been observed.

The differentiation of some of the cells of the integument into archegonial cells as noted previously by the author (3) in *Solanum Melongena* has not been observed in any other species. But the differentiation of a nucellar cell at the chalazal end of the ovule into a conspicuous cell seems to be very common in some of the species. This cell is often found to be bi-nucleate in *Brunfelsia*, where it attains a bigger size (figs. 18, 101, 117 and 118). The occurrence of two embryo-sacs in the same ovule separated by non-sporogenous tissue is probably one of the causes of this abnormality (fig. 136). Besides this, two archegonial cells in the meiotic prophase stage have been observed in almost all the species investigated. Moreover, two linear tetrads lying side by side have been observed in *Brunfelsia* (fig. 120) and it is not surprising to find two embryo-sacs in the same ovule. Hurst (10) has also observed the presence of two normal linear tetrads of megaspores in a tetraploid species of *Rosa mollis*.

Megasporogenesis

The archegonial cell directly functions as the megaspore mother cell in Solanaceæ. It enlarges at first and the cytoplasm becomes slightly vacuolated. The enlarged megaspore mother cell then gives rise by two successive divisions to a normal linear tetrad of four megaspores each being delimited from the other by a distinct wall. Both the meiotic mitoses seem to be normal in all the species. In a hybrid variety of *Petunia nyctaginiflora*, a very irregular type of distribution of the homologous chromosomes have been observed during the heterotypic division (fig. 93). Laggards also appear to be very common. In *Physalis peruviana* it has been observed that the disjunction of a pair of homologous chromosomes takes place earlier than the others (fig. 32). It cannot be said with certainty whether this is a constant feature

of this species. The haploid number of chromosomes counted during megasporogenesis corroborates the previous determination made by the author (4) from the microspore mother cells of the different species investigated. Although a detailed study of the method of chromosome conjugation has not been made during the present investigation it appears from the general behaviour of the nucleus during heterotypic prophase stages, that a telosynaptic mode of chromosome-conjugation is common in the different species. A typical second contraction stage with bivalent loops forming a loose knot, has been shown for *Cestrum diurnum* (fig. 63). In all the species that have been examined critically, it has been observed that the homotypic split appears in the univalent chromosomes during late anaphase and they appear as bivalents when they reach the poles (fig. 119). Although the megaspore mother cells vary in size in the different species, the heterotypic spindles are characteristically of the same size. The homotypic spindles generally orientate themselves similarly, but sometimes they have been observed to lie at various angles, with each other, with the result that the two pairs of megaspores lie in different planes.

The four megaspores when first formed are all alike in size and contents, but soon the chalazal megaspore begins to enlarge and its cytoplasm becomes markedly vacuolated. Generally the process of degeneration of the megaspores starts from the micropylar end down, though deviations from this are not uncommon. In some preparations of *Physalis* it has been observed that before the formation of a wall between the two megaspores at the micropylar end, the two nuclei show signs of degeneration, whereas the two megaspores at chalazal end remain normal (figs. 25 and 38). In *Lycopersicon esculentum* and in *Solanum nigrum* the development of the third and fourth megaspores from the micropylar end has been after observed (figs. 6 and 14). Similar abnormal development of the megaspores has been observed by Hurst (10) in a tetraploid form of *Rosa pomifera* and by the author (3) in *Solanum Melongena*. This probably is an additional cause of the development of two embryo-sacs in the same ovule.

The three disintegrated megaspores form a cap over the functioning one and in sections appear as crescents, triangles, or irregular masses. With the growth of the chalazal megaspore the degenerated megaspores are compressed and appear as a compact mass over it.

The Development of the Female Gametophyte

The resting nucleus of the functioning chalazal megaspore contains a thin lightly stained peripheral reticulum and a big nucleolus. With the initiation of its activity the whole cavity becomes filled up with a darkly stained embryo-sac. The two nuclei thus produced lie side by side for some time. The embryo-sac next increases in dimensions. The cytoplasm cannot keep pace with the developing embryo-sac and consequently vacuoles of varying dimensions originate in the cavity of the embryo-sac. A big central vacuole is ultimately formed by the fusion of the smaller vacuoles and the two nuclei are pushed to the two poles either by one side or through the middle of the embryo-sac. The next two mitotic divisions take place simultaneously but in one preparation of *Physalis minima* it has been observed that before the chalazal nucleus has divided the nucleus at the micropylar end has already divided (fig. 28). During the four nucleate stage the two nuclei at each pole, particularly the pair at the chalazal end, generally lie one above the other (figs. 7, 49, 68, 86 and 110). It appears that the orientation of the spindles during the next division and consequently the origin of the different elements of the mature embryo-sac is fixed at this stage (figs. 29, 39 and 97). During the next division there seems to be an aggregation of cytoplasm at either pole of the embryo-sac which foreshadows the formation of cells.

The third or the last division of the embryo-sac takes place very soon after the second division. In *Petunia nyctaginiflora*, *Brunfelsia americana* and *Datura fastuosa*, however, it has been observed that the last division takes place very late, sometimes after the opening of the flowers. It is interesting to note in this connection that ovaries of *Brunfelsia americana*, and in *Datura fastuosa*, fixed during the months of May and June, showed fully developed embryo-sacs, three or four days previous to the opening of the flowers. Considerable sterility of the female gametophyte and crumpling of the ovules have also been noted in this material (fig. 137). In material fixed during the months of September and October mature embryo-sacs were only found either in fully opened flowers or in buds which should open the next morning.

Of the four nuclei at the micropylar end of the embryo-sac, the two nearest to this end form the synergids. The egg is formed from one of the two remaining nuclei and is placed laterally between the synergids. The fourth nucleus forms the micropylar polar nucleus, and is placed below one of the synergids. Out of the four nuclei at the chalazal end of the embryo-sac the two nuclei derived from the spindle nearest to this end form the antipodal cells and occupy the chalazal groove of the embryo-sac. The third antipodal cell is derived from one of the remaining nuclei nearer to the chalazal end. The chalazal polar nucleus always lies just above the antipodals, at one side of the embryo-sac.

The synergids when first formed are triangular in outline with dense cytoplasm and conspicuous nucleus at the base (fig. 126). They begin to enlarge rapidly and become pear shaped. The nucleus is gradually pushed above due to the formation of a large vacuole at the base of each synergid. In all the species investigated the synergids have long acute beaks fitting into the micropylar end of the embryo-sac (figs. 15, 16, 50, 87 and 88). According to Coulter and Chamberlain (8) this is a characteristic feature of the Sympetalæ. The uniformity in the shape and size of the synergids suggests that they act as haustorial organs and probably help the unfertilized egg and the primary endosperm nucleus in their nutrition. They also appear to have a mechanical function in leading the pollen tube to the egg.

Cooper (7) in *Lycopersicum esculentum* has observed a well defined filiform apparatus similar to that observed by Miss Brown (5) in *Phaseolus*. Guignard (9), Svensson (17) and Young (19), however, have not described filiform apparatus for the species they have investigated. Well defined filiform apparatus has also not been observed in the course of this investigation. In *Physalis peruviana*, however, aggregation of cytoplasm in one or more strips at the apical region of the synergids have been observed (fig. 41).

With the growth of the embryo-sac, the egg enlarges and becomes more or less pear shaped in outline. Its basal region gradually widens apart on the synergids and its apical region protrudes beyond the synergids into the cavity of the embryo-sac. It contains a large vacuole near the base and its cytoplasm is dense at the apex. Young (19) states that the polar nucleus is scarcely as big as the synergid nucleus. From actual measurement given in table below it will be seen that the egg nucleus is smaller than the polar nucleus and its size is almost the same as the nucleus of the synergid.

The antipodals which occupy the chalazal groove of the embryo-sac are variable in their shape and arrangement in the same and different species (figs. 42, 69, 74, 89, 112, 122 and 123). Generally they are more or less triangular in outline. In some species (*Datura*, *Lycopersicum*, etc.) the two lower ones are long and rectangular and fit in the chalazal groove of the embryo-sac, while the third one is comparatively broader and lie above the two lower ones (figs. 125, 126, 127, 130 and 132). In *Cestrum*, however, the two antipodals have been found to lie above a broad basal antipodal cell (fig. 69). The arrangement of the antipodals seems

TABLE I

NAME OF THE PLANT.	Measurements of the average dimensions of the egg, polar and the synergid nuclei.		
	Egg nucleus.	Synergid nucleus.	Polar nucleus.
<i>Solanum nigrum</i> L. ..	4.8 μ	4.5 μ	6.3 μ
<i>Lycopersicum esculentum</i> Mill.	4.9 μ	4.8 μ	6.3 μ
<i>Physalis minima</i> L. ..	4.6 μ	4.5 μ	6.0 μ
<i>Physalis peruviana</i> L. ..	4.9 μ	4.5 μ	6.4 μ
<i>Withania somnifera</i> Dum. ..	4.5 μ	4.6 μ	6.0 μ
<i>Datura fastuosa</i> L. ..	5.8 μ	5.2 μ	7.0 μ
<i>Cestrum nocturnum</i> L. ..	7.6 μ	7.0 μ	10.2 μ
<i>Cestrum diurnum</i> L. ..	7.6 μ	7.0 μ	10.0 μ
<i>Nicotiana plumbaginifolia</i> Viv.	4.8 μ	4.8 μ	6.4 μ
<i>Petunia nyctaginiflora</i> Juss. ..	4.5 μ	3.5 μ	6.7 μ
<i>Salpiglossis sinuata</i> Ruiz. ..	4.6 μ	4.6 μ	6.4 μ
<i>Brunfelsia americana</i> Sw. ..	5.4 μ	4.9 μ	7.5 μ

to depend on the plane of orientation of the spindles at the chalazal end of the embryo-sac. The antipodals are as a rule ephemeral organs. According to Schnarf (14) the antipodals are big and uninucleate in *Solanum dulcamara*, *Atropa Belladonna*, and in *Nicotiana Tabacum*. In *Hyoscyamus niger*, and in *Datura laevis* they are small and degenerate early. In *Datura metel* they persist long after fertilization. From the evidence obtained during this investigation it appears that the antipodal cells degenerate mostly at the time of fertilization. In *Datura*, *Lycopersicum* and *Nicotiana* their outline could be seen after fertilization. Soueges (16) believes that the chalazal groove of the embryo-sac is a haustoria and the antipodal cells act as secretory organs which actively secrete chemical substances and help in digesting the nucellar tissue behind, thus forming the "chalazal pocket" commonly met with in most of the species. The results obtained during this investigation lead us to believe that the chalazal groove plays the role of haustoria and absorbs nourishment through its entire surface, the digestive process being brought out by the antipodals during pre-fertilization stages, and later on by the endospermic cells at the chalazal end of the embryo-sac.

The polar nucleus from the chalazal end of the embryo-sac migrates towards the micropylar end and meets the other polar nucleus generally just by the side, or below the egg. Sometimes, however, they meet lower down the embryo-sac. In some preparations they have been observed to lie at one side of the embryo-sac, but this might be due to imperfect fixation. In fully mature embryo-sac, however, the two polar nuclei, or the fused polar nucleus, have always been observed to lie very close to the egg. The two polar

nuclei lie close together for some time and then fuse. From a comparative study of the fusion of the polar nuclei in all the species we have come to the conclusion that in the same species, the fusion of the polar nuclei may take place either before the opening of the flower, or even after the entrance of the pollen tube.

The mature embryo-sac is surrounded by a thin layer of cytoplasm, excepting the two poles where the cytoplasm is very dense. Vacuoles of varying dimension appear between the poles. In *Cestrum*, an accumulation of starch grains within the embryo-sac has been observed. They are particularly abundant near the primary endosperm nucleus and are very conspicuous (figs. 72, 73 and 124). Along with the maturity of the female gametophyte the single layer of nucellar cells degenerate completely and during later stages the fully developed gametophyte lies adpressed to the tapetal tissue.

Tapetal Tissue

The innermost layer of cells of the integument begin to differentiate at about the time the megaspore mother cell initiates activity. This differentiation starts simultaneously from the micropylar, as well as from the chalazal end of the embryo-sac. This "tapetal" or nutritive tissue consists of rectangular cells with their long axes perpendicular to the long axis of the embryo-sac. The cells contain dense cytoplasm and conspicuous nuclei. Exceptionally, however, bi-nucleate tapetal cells have been observed in some preparations. Svensson (17) has observed starch kernels in the tapetal cells of *Hyoscyamus niger* which, however, have not been observed during this investigation. The cells of the tapetal tissue divide mitotically, but always remain a single layer of cells.

Discussion

Development of ovule and integument:—

A comparative study of the flowers in the different species of Solanaceæ shows that numerous ovules are borne, as a rule on a massive placenta. Due to the development of lateral outgrowth from this axile peltate placenta the bi-celled ovary common in Solanaceæ often becomes three to six celled, as in *Lycopersicum esculentum*, *Datura fastuosa* and others. The presence of this massive placenta has been described by Hooker and Engler as one of the characteristic features of the flowers of Solanaceæ. The origin of the ovules from the placenta, so far the author is aware, has not been fully described by previous authors. From this investigation it appears that the origin of the ovules follows a definite process. The subsequent development of the ovules, however, varies in some of the genera. A transition from anatropous to campylotropous form of the ovule can be seen also in the different genera. Chatin (6) found anatropous ovules in *Nicotiana rustica*, *Nicotiana Tabacum* and *Datura Stramonium*. Cooper (7) has also described the ovules of *Lycopersicum esculentum* as anatropous. Young (19), however, is of opinion that the ovules in *Solanum tuberosum* have not the typical anatropous form, since the embryo-sac has been found to be considerably curved. He has suggested that this is a transitional stage towards campylotropy. It has been pointed out before, that the ovules are strictly campylotropous in *Cestrum* and anatropous in

Nicotiana and *Petunia*. In the remaining genera they are not strictly anatropous, for the long axis of the embryo-sac has never been observed to be perfectly vertical. It seems that these intermediate forms are transitional stages from anatropy to campylotropy. It is also very interesting to note that this difference in the orientation of the ovules is only present in the tribe Cestreæ. It will be seen from the text that the genus *Cestrum* has some characters which does not confirm to the general plan of organisation met with in the different genera of Solanaceæ. Specially in *Petunia* and *Nicotiana*, which are members of the same tribe, Cestreæ.

The process of development of the integument is also very uniform in all the species. The histological differentiation of the integument, however, is very different in the different genera as in some of the species of *Solanum*. Soueges (16) has classified the principal genera of Solanaceæ into two broad groups, based on the persistence or non-persistence of the digestive layer, the "assise digestive". The subsequent subdivisions, however, depend on minute details. Regarding this character also it will be seen that in *Cestrum* this digestive layer persists after fertilization whereas in *Petunia* and *Nicotiana* it degenerates. The integument is massive in all the species as is common in members of the Sympetalæ, forming a long micropyle.

Development of the female gametophyte:—

Early investigators have found a normal type of development of the female gametophyte in Solanaceæ. Nanetti (12) in *Solanum muricatum* and Young (19) in *Solanum tuberosum* on the contrary have found "Lilium type" of development of the embryo-sac. The author has found on the contrary a normal type of development in *Solanum Melongena*. Recently Cooper (7) has recorded also a normal type of development in *Lycopersicum esculentum*. In the present investigation it has been found that the development of the female gametophyte is of the normal type in all the species, and considering the previous work on the subject we are inclined to believe that Young's and Nanetti's observations were probably inaccurate. Schnarf (14) is also of opinion that a reinvestigation is necessary before accepting Young's and Nanetti's observations.

Tapetal tissue:—

The presence of a well differentiated tapetal tissue covering the embryo-sac is not frequently met with as compared to its constant presence in the microsporangium of higher plants. Soueges (16), however, has shown that this tapetal tissue (covering the embryo-sac) is a characteristic feature of the different species of Solanaceæ. During the present investigation, the differentiation of the tapetal tissue from the integument has been observed in all the species. This tissue plays an important part in storing food material in the

endosperm cells and thus indirectly nourishes the future sporophyte; the food stuff being derived from the digested integumental cells. This tissue, therefore, serves the double function of secretion and absorption. Mascré (11) has shown that a typical secretion tapetum covering the microsporangium is also a characteristic feature, commonly met with in this family. A nutritive fluid is secreted by this tapetum by a process of caryolysis for the nutrition of the developing pollen mother cells. Along with this phenomena, the tapetal cells undergo degeneration with which is associated some degenerative changes in the contents of the cells. Deeply staining globular bodies either scattered, or in groups, have been described as products of fat degeneration. They begin to accumulate together with mitochondric granules. The first sign of degeneration, however, can be seen in the cytoplasm, which becomes very much vacuolated. The single nucleus divides and forms typical bi-nucleate tapetal cells, common in plants. These two nuclei may again fuse and become uninucleate. Sometimes they divide once or twice and form four to six nucleate tapetal cells. The process of development of the tapetum has not been presented in the text as the observations have been found to corroborate Mascré (11) in every detail. From a comparative study of all the species it seems that bi-nucleate tapetal cells covering the embryo-sac, which has been found to be very common in *Solanum Melongena*, however, is rarely met with in other species. Cytological changes in the contents of the tapetal cells as found in the microsporangium, however, have not been observed. The difference seems to be clear from the part played by the tapetal cells in supplying nutrition to the gametophytes in either case. In the microsporangium the nutrition is derived from the tapetal cells themselves, and is necessary only for a short period of time. In the ovules on the contrary, the nutrition is derived from the integumental cells, the tapetum only makes the nutrition absorbable for the endosperm cells. In the latter case also, the nutrition is necessary for a longer period of time.

Degeneration of the ovules:—

In most of the flowering plants, excepting the cleistogamic forms, the female gametophyte becomes fully differentiated before the opening of the flower. As mentioned before in some species of Solanaceæ the embryo-sac does not attain full maturity before opening of the flowers. This phenomenon, however, does not appear to be a specific feature of any species. On the contrary maturity is somewhat dependent on the temperature and relative humidity prevailing during anthesis. Crumpling of the ovules leading to the failure of seed development has been noted in *Datura fastuosa*, *Brunfelsia americana*, and *Nicotiana plumbaginifolia* during hot and dry seasons. Smith (15) working on *Lycopersicum esculentum* has recorded the percentage of blossom drop in relation to the atmospheric temperature. She has shown that the temperature prevailing three days before anthesis has the greatest influence

on it. From the evidence obtained during the course of this investigation it appears that this degeneration is due to failure in the development of the pollen tubes in large numbers on the stigma. Further experiments in this line are necessary for a proper understanding of the phenomenon.

Filiform apparatus:—

The presence of a well defined filiform apparatus has not been recorded by previous investigators in any species of this family. Cooper (7), however, has recently recorded a well defined filiform apparatus in *Lycopersicum esculentum*. No such filiform apparatus in the synergids of *Lycopersicum* was noted during this investigation. From the general absence of the structure in all the species, it seems that Cooper's observation cannot be corroborated.

Antipodals:—

The antipodal cells are variable in shape, in the different genera and even in the same species. They are very big in *Cestrum*, unlike those occurring in other species. The degeneration of the antipodals or their persistence until fertilization, or a little longer does not appear to be a fixed character for any species. Cooper (7) for example, observed the degeneration of the antipodal cells in *Lycopersicum* before fertilization. During the present study the antipodals have been found in some preparations of *Lycopersicum* persisting, even when the endosperm nucleus has divided once or twice.

Content of the mature embryo-sac:—

The presence of starch grains in the embryo-sac has not been observed by previous investigators in this family. Svensson (17) has observed starch kernels in the tapetal cells covering the embryo-sac, in *Hyoscyamus niger*. The presence of starch grains in the embryo-sac of *Cestrum* and their abundance round the polar fusion nucleus, shows an efficient method of supplying nutrition to the nucleus at the time of fertilization.

Abnormalities:—

A number of abnormalities in the development of the embryo-sac have been described in the text. More than one archesporial cells in the same nucellus have been previously recorded by Young (19) in *Solanum tuberosum* and by Svensson (17) in *Hyoscyamus niger*. Cooper (7), however, has not mentioned it in *Lycopersicum*. During the present study more than one archesporial cells have been observed in most of the species. In *Lycopersicum* also, two and sometimes more than two archesporial cells have been observed to differentiate out in the nucellus. Similar multiple archesporium has also been recorded by the author (3) in *Solanum Melongena*. From the present study it appears that the origin of the multiple archesporium is due to the subsequent division of one or two hypo-

dermal archesporial cells. Two archesporial cells have been also found developing as megaspore mother cells in most of the species. Their development up to the tetrad stage has been noted in *Brunfelsia*. Hurst (10) has observed similar development in *Rosa mollis*. It is not unlikely that they develop further, forming more than one embryo-sac in the same ovule as has been observed during this investigation in *Withania somnifera*, *Physalis minima* and *Nicotiana plumbaginifolia*. Hurst (10) has also observed the development of more than one embryo-sac in another species of *Rosa*. It has been also described that the third megaspore from the micropylar end also develop in some cases along with the chalazal one. Afzelius (1) observed similar phenomenon in certain species of *Senecio* and allied genera, which has also been recorded by Hurst (10) in *Rosa pomifera* and by the author (3) in *Solanum Melongena*. The subsequent development of the third megaspore could not be traced, but it seems quite likely that it produces an additional embryo-sac in the same ovule.

The development of a chalazal nucellar cell, as a megaspore mother cell, up to the linear tetrad stage has been recorded by the author (3) in *Solanum Melongena*. In the case of *Brunfelsia* it seems that this abnormal type of development of a chalazal nucellar cell does not follow the "normal type" but probably the "Lilium type," since no partition wall has been observed during the bi-nucleate condition. The embryo-sac assumes the bi-nucleate stage from this stage. Further development has been followed only upto four-nucleate stage and it cannot be said definitely whether a normal embryo-sac is formed or not.

Summary

1. The origin of the ovule from the placenta has been traced in all the species and their development found to be very uniform throughout this family.
2. The origin and the development of the integument in the various species of Solanaceæ studied corroborates Soueges' observations. A transition from anatropous to campylotropous form of the ovule has been noted in the tribe Cestreæ.
3. The archesporial cell differentiates at the time of differentiation of the integument and is of hypodermal origin. More than one archesporial cells have been noted in most of the species.
4. The meiotic divisions of the megaspore mother cells appear to be normal in all the species. In one hybrid form of *Petunia* irregularity in the distribution of the homologous chromosomes was noted.
5. The development of the female gametophyte in the following species have been determined for the first time:—*Solanum*

nigrum, *Physalis minima*, *Physalis peruviana*, *Withania somnifera*, *Datura fastuosa*, *Cestrum nocturnum*, *Cestrum diurnum*, *Nicotiana plumbaginifolia*, *Petunia nyctaginiflora*, *Salpiglossis sinuata* and *Brunfelsia americana*. A "normal type" of development of the female gametophyte has been noted in all the species.

6. The synergids in all the species have long acute beaks and they function as haustoria.

7. The dimension of the egg is constant in all the species except in *Cestrum*.

8. The fusion of the polar nuclei generally takes place before opening of the flower, though it may be delayed in some cases until the entrance of the pollen tube.

9. The antipodal cells are variable in shape and size and usually degenerate at the time of fertilization. They function as haustoria and help in supplying nutrition to the polar nuclei and the egg before fertilization.

10. The maturity of the embryo-sac seems to be partly dependent on the atmospheric temperature and humidity. The crumpling of the ovules and the non-setting of the seeds are due to low percentage of germination of pollen grains on the stigma. This phenomenon seems to be also associated with unfavourable atmospheric conditions.

11. A well differentiated tapetal tissue covering the embryo-sac has been observed in all the species. The physiological function and the mechanism of supplying nutrition to the embryo have been followed in some of the species.

12. The presence of starch grain in the cytoplasm of the mature embryo-sac of *Cestrum* has been noted.

13. Development of more than one megaspore mother cells appears to be very common in this family. Their development up to the tetrad stage has been observed in *Brunfelsia*. More than one embryo-sac in the same ovule has been found in *Withania*, *Physalis*, *Nicotiana* and *Brunfelsia*.

In conclusion I desire to express my thanks to Mr. I Banerji for his helpful suggestions and continued interest during the progress of this investigation.

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Explanation of Plates

Plate I. Figs. 1-7. Showing stages in the development of the ovule and embryo-sac in *Solanum nigrum* L.

1. Two archesporial cells one below the other. 2. Two megaspore mother cells in meiotic prophase stage. 3. Dyad stage, just formed. 4. Homotypic division, showing also the development of the integument in relation to megasporogenesis. 5. Three disintegrated megaspores on the functioning chalazal one. 6. Third and fourth megaspores developing simultaneously. 7. Four nucleate embryo-sac. $\times 500$.

Plate II. Figs. 8-17. Showing stages in the development of the ovule and embryo-sac in *Lycopersicon esculentum* Mill.

8-10. Development of more than one archesporial cells in the same ovule $\times 850$. 11. Dyad stage, note the differentiation of the tapetal tissue. 12. Normal linear tetrad. 13. The disintegrated megaspores forming a cap over the functioning chalazal one. 14. The third and the fourth megaspore developing simultaneously. 15-16. The egg apparatus and the fused polar nuclei. Note the absence of filiform apparatus in the synergids. $\times 850$. 17. Late heterotypic anaphase, polar view, showing the 12 haploid chromosomes. $\times 3,500$. $\times 500$.

Plate III. Figs. 18-25. Showing stages in the development of the ovule and embryo-sac in *Physalis minima* L.

18. Early stage in the development of the ovule. Note the bi-nucleate chalazal nucellar cell. 19-20. Single and double archesporial cells. 21. Diakinesis stage and the development of the integument. 22. Heterotypic metaphase. 23. Dyad stage. 24. Normal linear tetrad. 25. The third megaspore from the micropylar end degenerating before the upper two megaspores are well formed. $\times 500$.

Plate IV. Figs. 26-32. Showing stages in the development of the ovule and embryo-sac in *Physalis minima* L.—(contd.) and *P. peruviana* L.

26-27. Disintegration of the functionless megaspores and the development of the functioning chalazal one. 28. Three nucleate embryo-sac. 29. Four nucleate embryo-sac dividing to form eight nucleate stage. Note the orientation of the spindles. 30. Mature embryo-sac, the antipodals are degenerating. 31. Early stage in the development of the ovule and the differentiation of the archesporial cell in *Physalis peruviana*. 32. Early separation of a pair of chromosomes during heterotypic anaphase. Note also the deeply buried megaspore mother cell and the tapetal tissue. $\times 500$.

Plate V. Figs. 33-42. Showing stages in the development of the ovule and embryo-sac in *Physalis peruviana* L.—(contd.)

33. Three archesporial cells. 34. Two archesporial cells, one below the other. 35. Two megaspore mother cells in early meiotic prophase stage. 36. Open spireme stage. 37. Dyad stage. 38. The upper two megaspores from the micropylar end showing signs of degeneration before they are well formed. $\times 850$. 39. Eight nucleate embryo-sac just formed. Note the orientations of the nuclei. 40. The three degenerated megaspores forming a cap over the functioning chalazal one. 41. Two fully developed synergids. Note the striated appearance of the cytoplasm at the apical region of each synergid. $\times 1,100$. 42. The differentiation of the antipodals at the chalazal groove of the embryo-sac. $\times 850$. $\times 500$.

Plate VI. Figs. 43-50. Showing stages in the development of the ovule and embryo-sac in *Withania somnifera* Dun.

43. Early stage in the development of the ovule and the differentiation of the hypodermal archesporial cell. 44. Two archesporial cells arranged side by side. 45. Heterotypic metaphase stage. 46. Late homotypic divisions. 47. Normal linear tetrad. 48. Disintegrated megaspores forming a cap over the functioning chalazal one. 49. Four nucleate embryo-sac. 50. Egg apparatus and the fused polar nucleus. $\times 500$.

Plate VII. Figs. 51-60. Showing stages in the development of the ovule and embryo-sac in *Datura fastuosa* L.

51. Early stage in the development of the ovule and the differentiation of the archesporial cell. 52. Leptonema stage. 53. Two megaspore mother cells in early meiotic prophase stage. 54. Heterotypic metaphase stage. 55-56. Homotypic divisions. 57-58. Disintegrated megaspores forming cap over the functioning chalazal one. 59. Heterotypic metaphase stage, polar view, showing 12 haploid chromosomes. $\times 3,500$. 60. First division of the chalazal megaspore. $\times 500$.

Plate VIII. Figs. 61-62. Showing stages in the development of the ovule and the differentiation of the archesporial cells in *Cestrum diurnum* L. and *Cestrum nocturnum* L., respectively. $\times 500$.

Plate IX. Figs. 63-69. Showing stages in the development of the embryo-sac in *Cestrum diurnum* L.

63. Megaspore mother cell in typical second contraction stage, note the bivalent loops. 64. Dyad stage. 65. Late homotypic division. 66. Disintegration of the functionless megaspores over the functioning chalazal one. 67. Bi-nucleate embryo-sac just formed. 68. Four-nucleate embryo-sac. 69. Three antipodal cells. $\times 850$. $\times 500$.

Plate X. Figs. 70-74. Showing stages in the development of the embryo-sac in *Cestrum nocturnum* L.

70. Homotypic division. 71. Disintegrated megaspores as a cap over the germinating chalazal one. 72. Egg apparatus and the fusion of the polar nuclei, note the conspicuous starch grains. 73. Mature embryo-sac with ovule. Note the campylotropous form of the ovule and the starch grains. $\times 180$. 74. Three antipodal cells. $\times 850$. $\times 500$.

Plate XI. Figs. 75-90. Showing stages in the development of the ovule and embryo-sac in *Nicotiana plumbaginifolia* Viv.

75. Early stage in the development of the ovule with single hypodermal archesporial cell. 76-77. Double archesporial cells. 78. Two archesporial cells developing simultaneously. 79. Megaspore mother cell in open spireme stage. 80-81. Heterotypic meta and telophase stages of the megaspore mother

cell. 82. Homotypic division. 83. Normal linear tetrad. 84. Three disintegrated megaspores forming a cap over the functioning chalazal one. 85. First division of the chalazal megaspore. 86. Four-nucleate embryo-sac, note the conspicuous tapetal cells. 87-88. Egg apparatus and the fusion of the polar nuclei. 89. The octonucleate embryo-sac. 90. Polar view, heterotypic metaphase stage of the megaspore mother cell, showing the haploid chromosome number ($n=10$). $\times 3,500$. $\times 500$.

Plate XII. Figs. 91-99. Showing stages in the development of the ovule and embryo-sac in *Petunia nyctaginiflora* Juss.

91. Early stage in the development of the ovule with single hypodermal archesporial cell. 92. Dyad stage. Note the development of the integument and the funiculus. 93. Irregular distribution of chromosomes during first meiotic mitosis of the megaspore mother cell in a hybrid variety of *Petunia*. $\times 1,100$. 94. Normal linear tetrad. 95. Disintegration of the functionless megaspores. 96. First division of the chalazal megaspore. 97. The four nuclei dividing to form eight nucleate embryo-sac note the orientation of the spindles. 98. Octonucleate embryo-sac, the polar nuclei have already fused, note also the disintegrated antipodal cells. 99. Oblique polar view, heterotypic metaphase stage of the megaspore mother cell showing the haploid chromosome number ($n=7$). $\times 3,500$. $\times 500$.

Plate XIII. Figs. 100-112. Showing stages in the development of the ovule and embryo-sac in *Salpiglossis sinuata* Ruiz.

100-102. Early stages in the development of the ovule, note the double archesporial cells. 103. Two megaspore mother cells side by side, developing simultaneously. 104. Heterotypic metaphase stage of the megaspore mother cell. 105. Dyad stage. 106. Homotypic divisions. 107. Disintegration of the functionless megaspores. 108. The disintegrated megaspores forming a cap over the functioning chalazal one. 109. First division of the chalazal megaspore. 110-111. Two and four nucleate embryo-sacs in divisional stages going to form four and eight nucleate stages respectively. Note the orientation of the spindles. 112. The octonucleate embryo-sac, the polar nuclei have not yet migrated. $\times 500$.

Plate XIV. Figs. 113-123. Showing stages in the development of the embryo-sac in *Brunfelsia americana* Sw.

113-115. One and more than one archesporial cells in the same ovule. 116. Two megaspore mother cells developing simultaneously. 117-118. The bi-nucleate chalazal nucellar cells. 119. Dyad stage. 120. Two normal linear tetrad arranged side by side in the same ovule. 121. The disintegrated megaspores forming a cap over the functioning chalazal one. 122. The three antipodal cells and one polar nucleus at the chalazal end of the embryo-sac. 123. Two antipodal cells persisting while the third one has disintegrated. $\times 500$.

Plate XV. Figs. 124-129. Showing mature embryo-sacs, form of the ovules and the nature of the integuments in the different species of Solanaceæ. (Reduced to half of the original size in the reproduction).

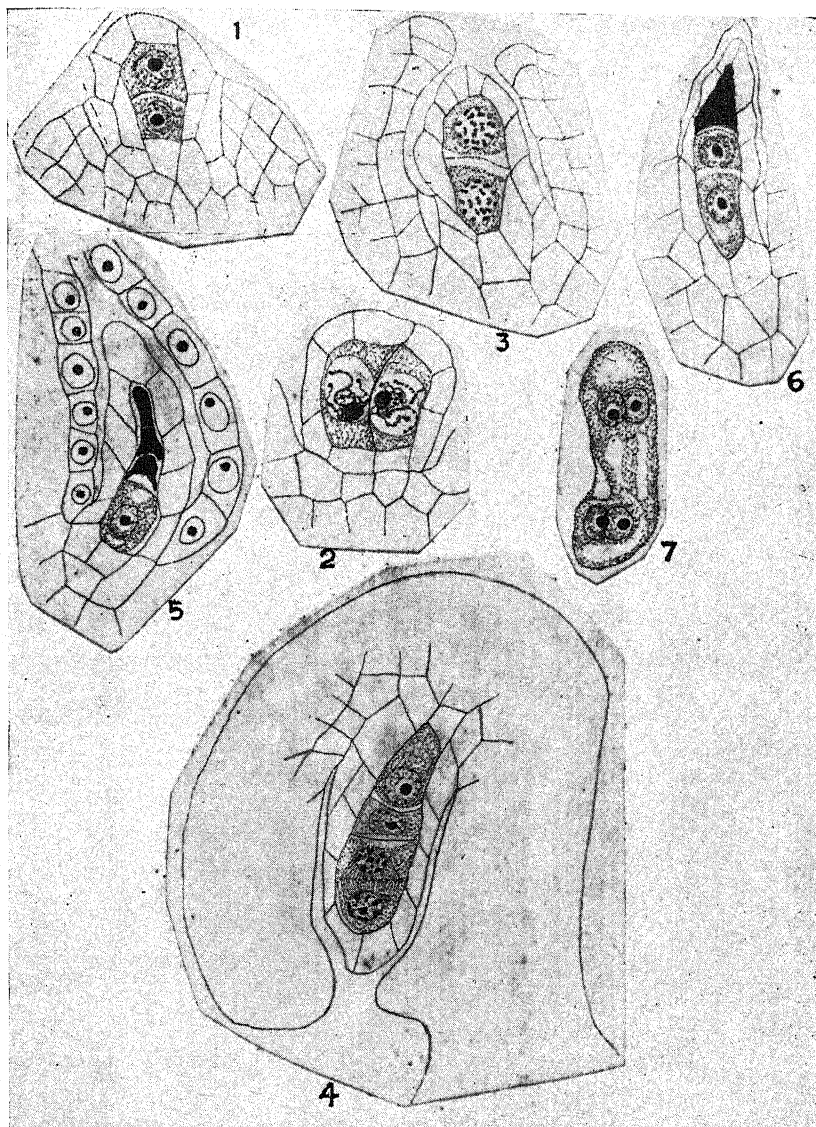
124. Mature embryo-sac of *Cestrum diurnum* L. Note the campylotropous form of the ovule and the conspicuous starch grains in the embryo-sac. $\times 400$. 125. Mature embryo-sac of *Datura fastuosa* L. 126. Mature embryo-sac of *Lycopersicum esculentum* Mill. Note the tapetal cells and the half anatropous form of the ovule. 127. Mature embryo-sac of *Physalis minima* L. Note also the half anatropous form of the ovule. 128. Mature embryo-sac of *Physalis peruviana* L. Note the massive integument. 129. Mature embryo-sac of *Solanum nigrum*. $\times 500$.

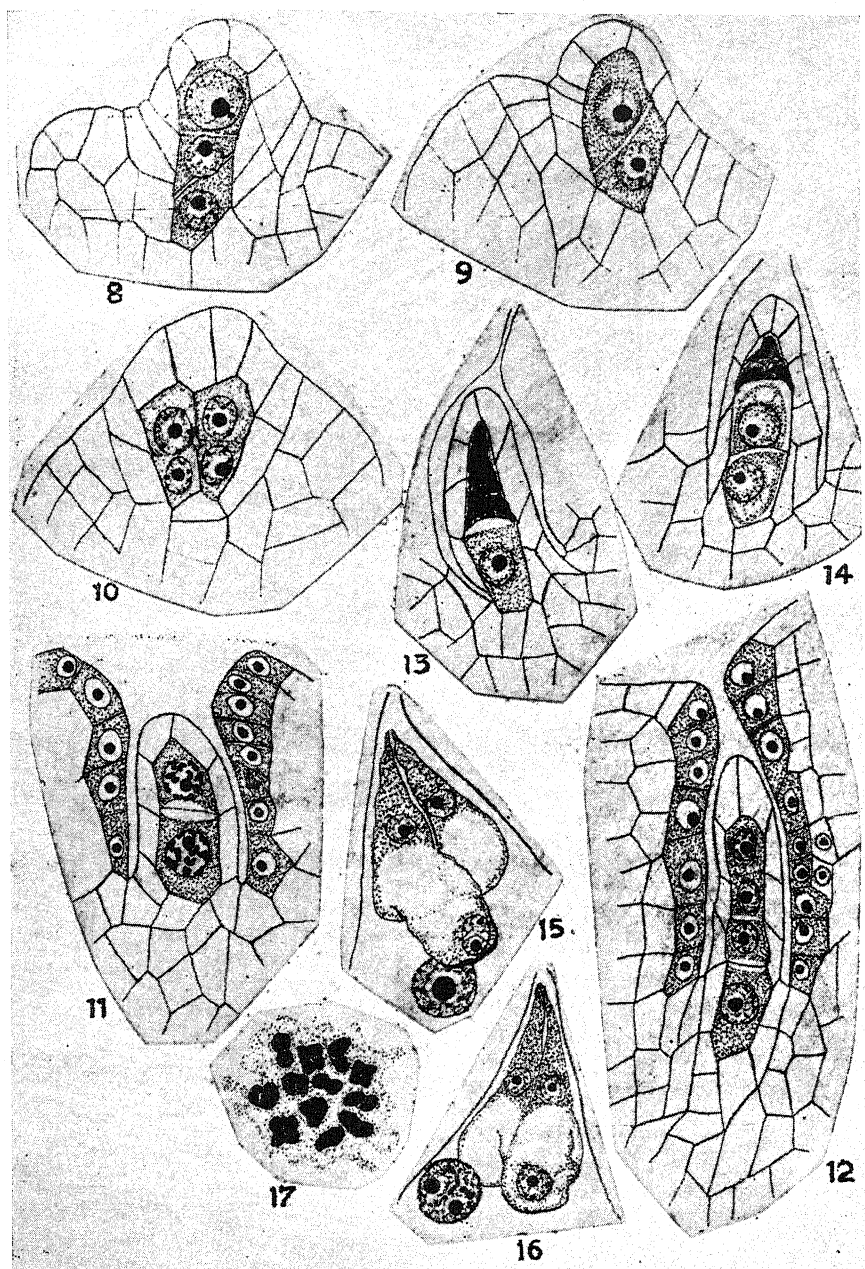
Plate XVI. Figs. 130-134. Showing mature embryo-sacs, form of the ovules and the nature of the integument in the different species of Solanaceæ. (Reduced to half of the original size in the reproduction).

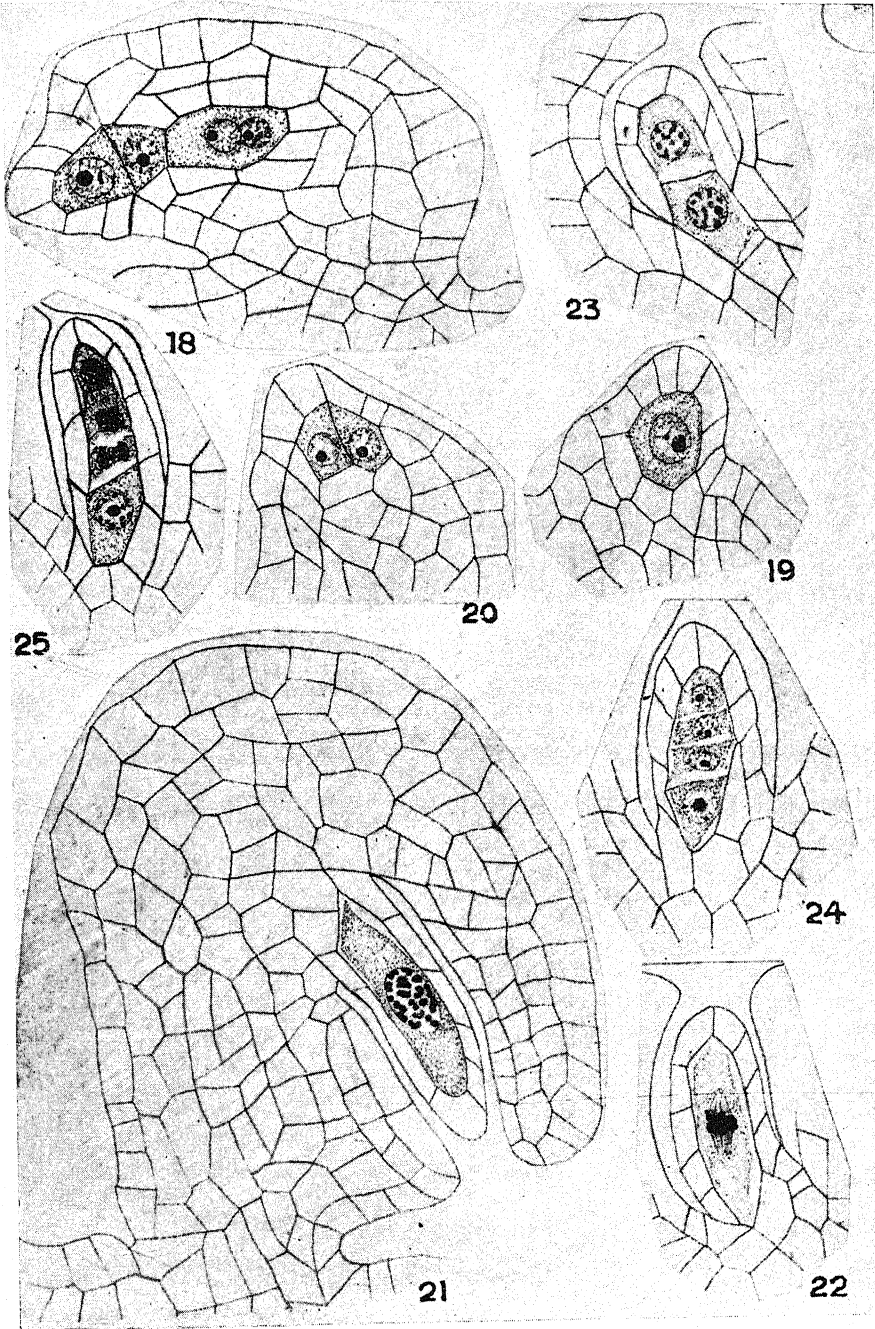
130. Mature embryo-sac of *Withania somnifera* Dun. 131. Mature embryo-sac of *Nicotiana plumbaginifolia* Viv. Note the anatropous form of the ovule. 132. Mature embryo-sac of *Petunia nyctaginiflora* Juss. Note also the perfect anatropous form of the ovule. One of the antipodal cells has probably divided forming four antipodal cells. 133. Mature embryo-sac of *Brunfelsia americana* Sw. Note the nucellar cells have protruded inside the cavity of the embryo-sac. 134. Mature embryo-sac of *Salpiglossis sinuata* Ruiz. $\times 500$.

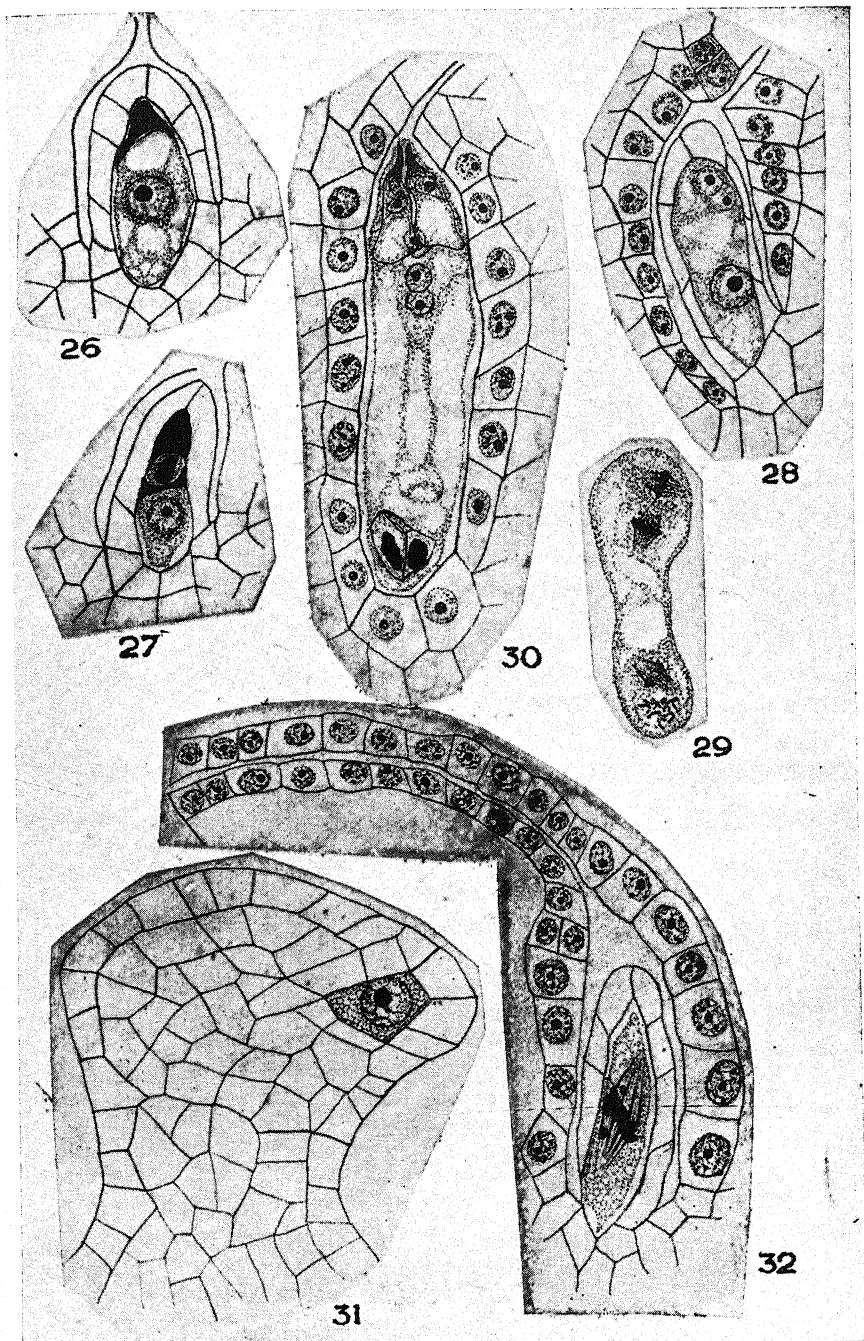
Plate XVII. Figs. 135-137. 135. Photomicrograph of a longitudinal section of the ovary of *Salpiglossis sinuata* Ruiz, showing the differentiation of the "ovule initials" in large numbers from the hypodermal and subhypodermal layers of cells.

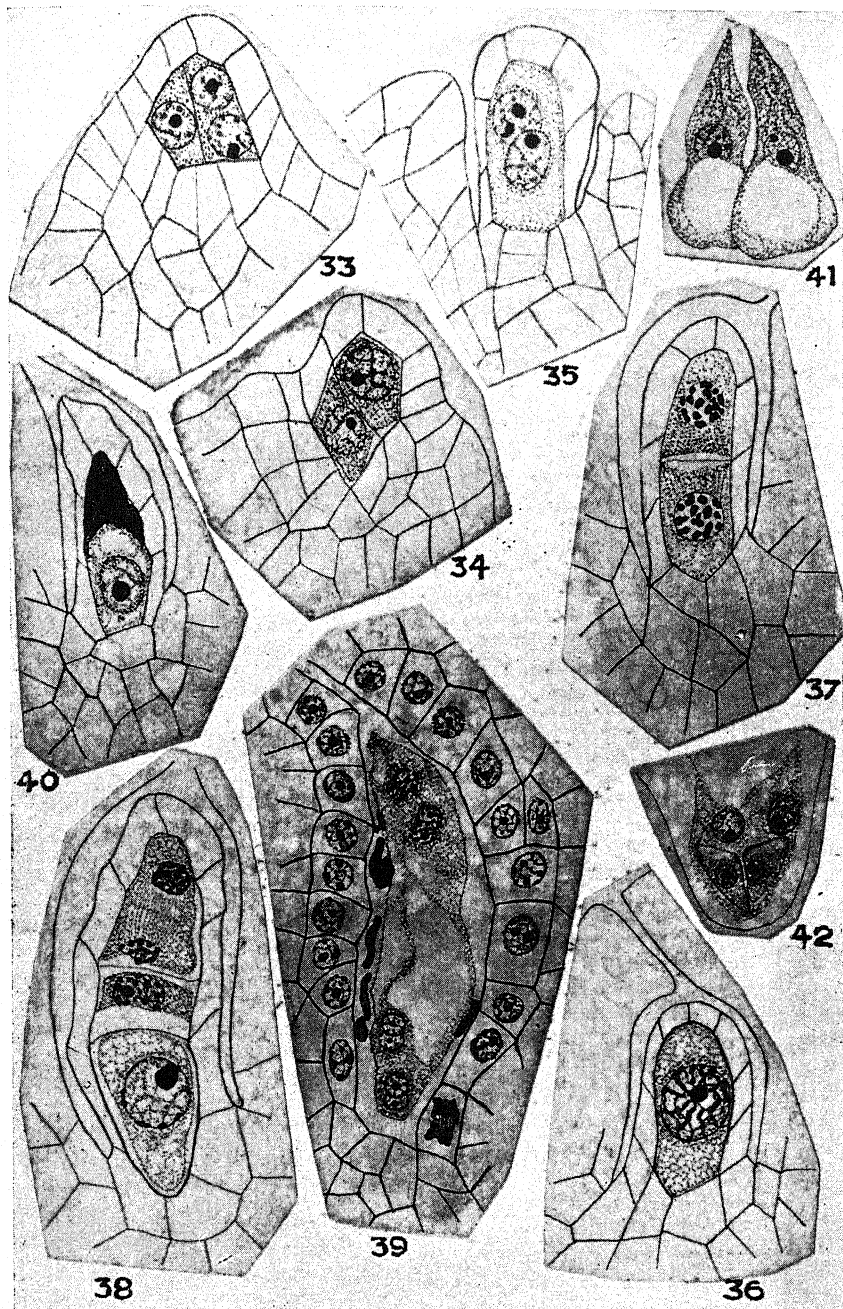
136. Photomicrograph of a longitudinal section of an ovule of *Physalis minima* L., showing two embryo-sacs in the same ovule separated from each other by differentiated tapetal tissue. Note that endosperm cells are well formed in one of the embryo-sacs. 137. Photomicrograph of a longitudinal section of an ovary of *Brunfelsia americana* Sw. (material fixed during the month of May, 1932), showing the crumpling and sterility of the ovules.



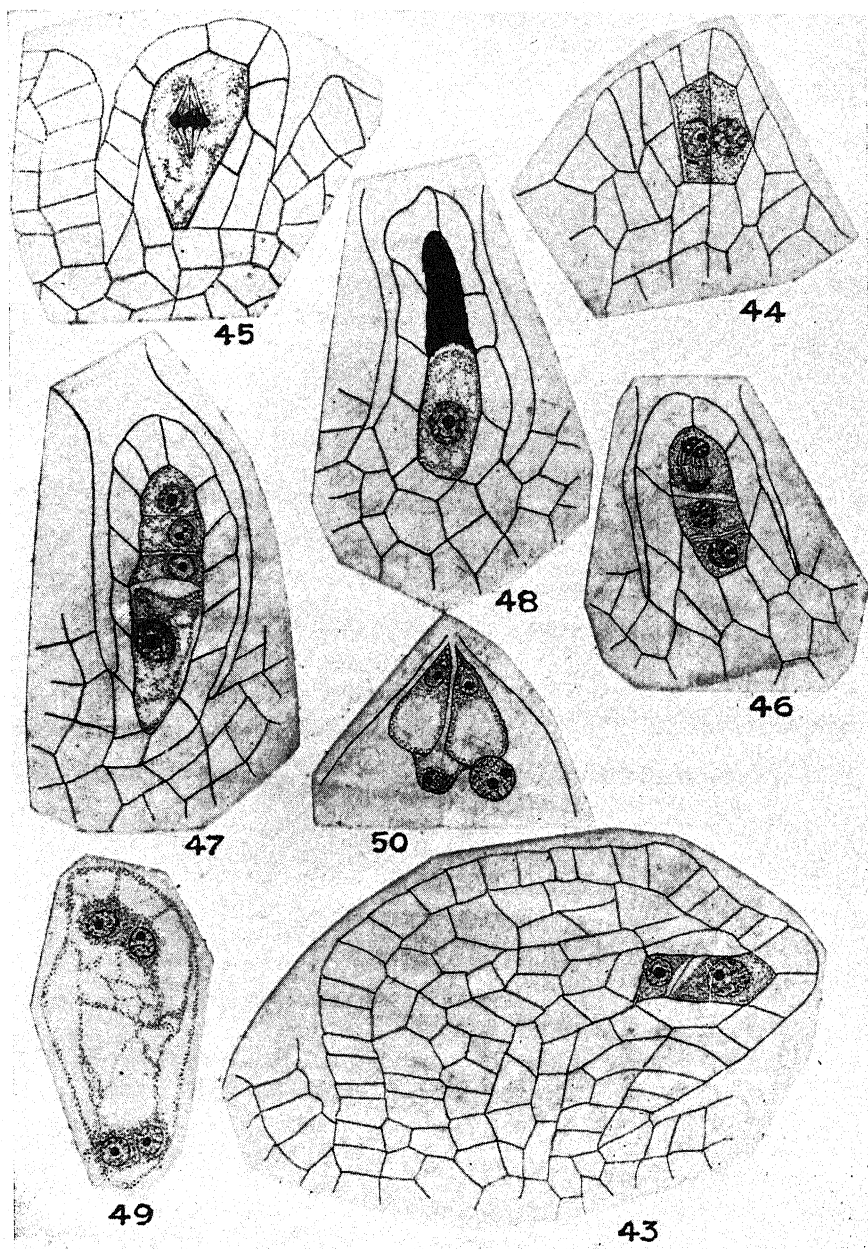


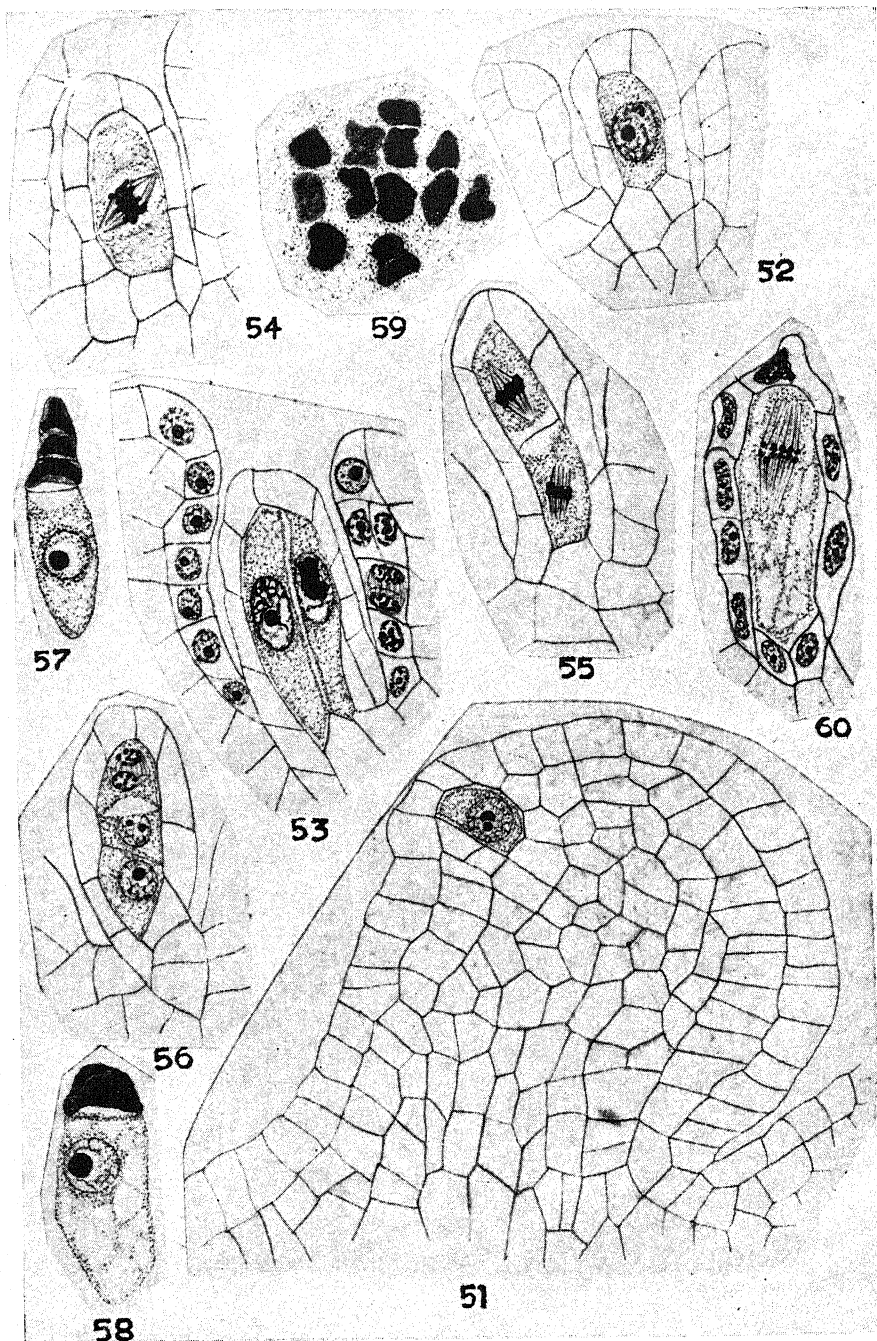


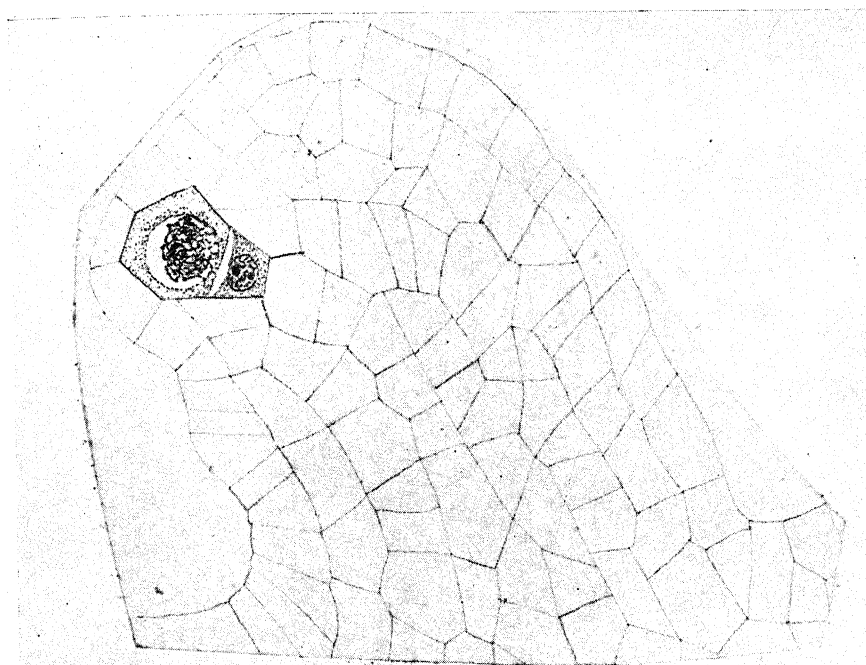




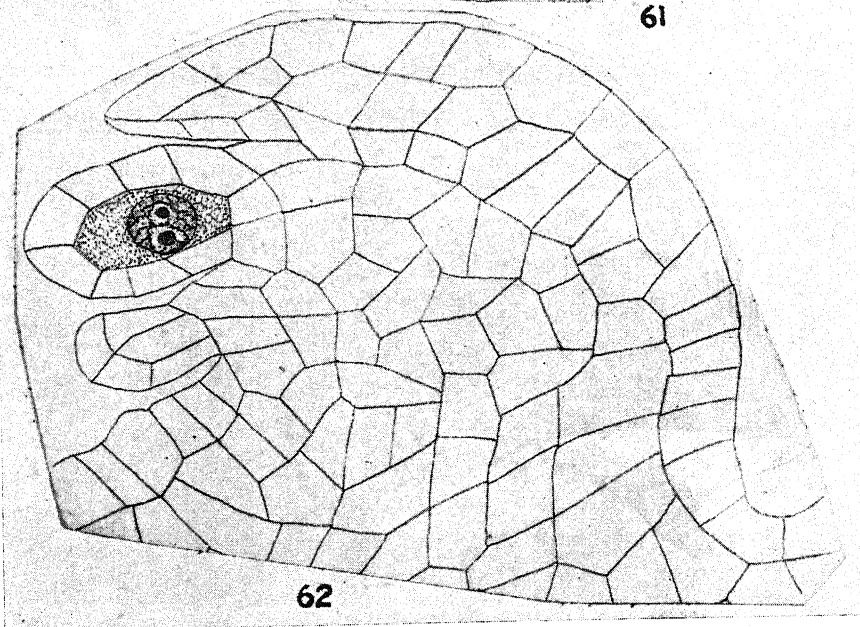






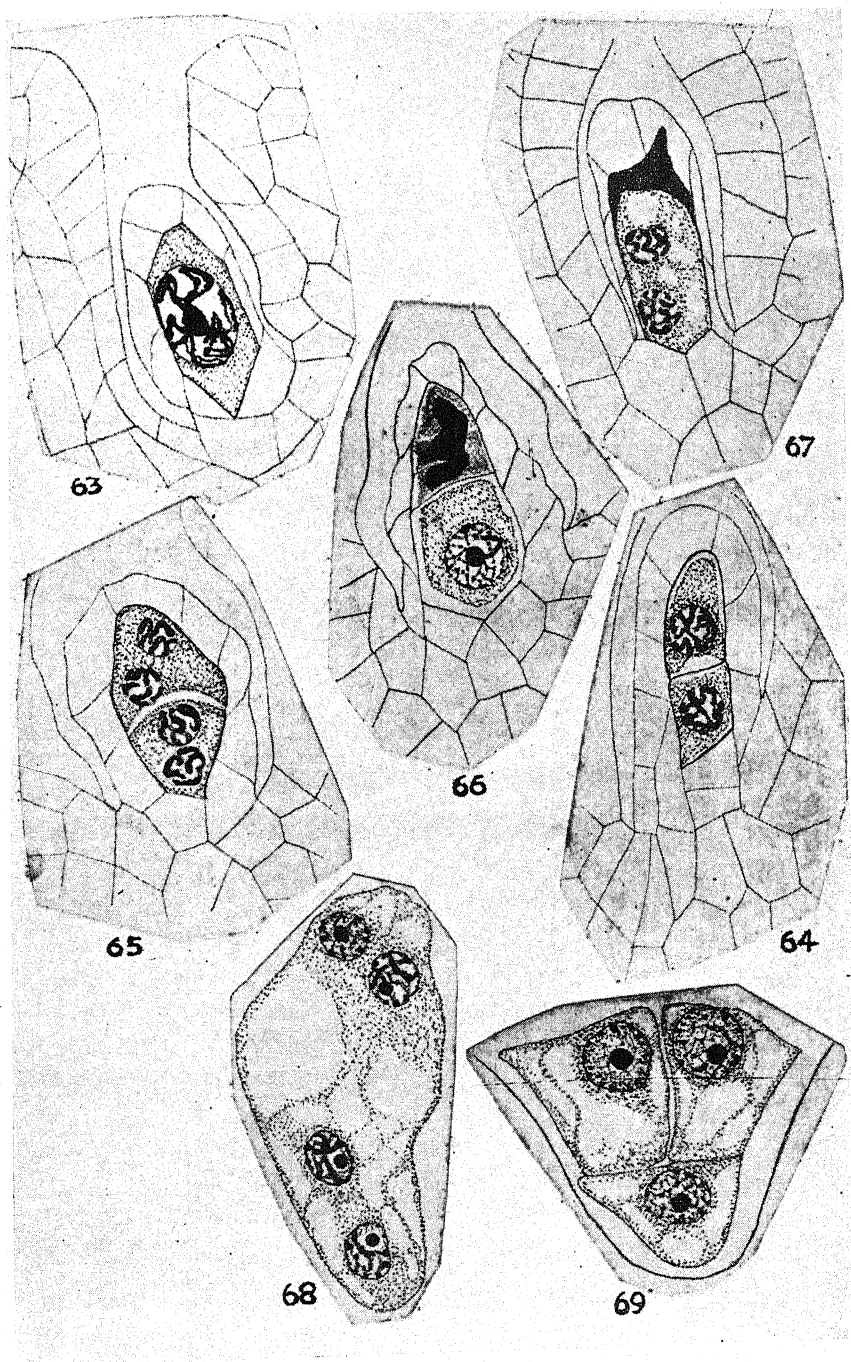


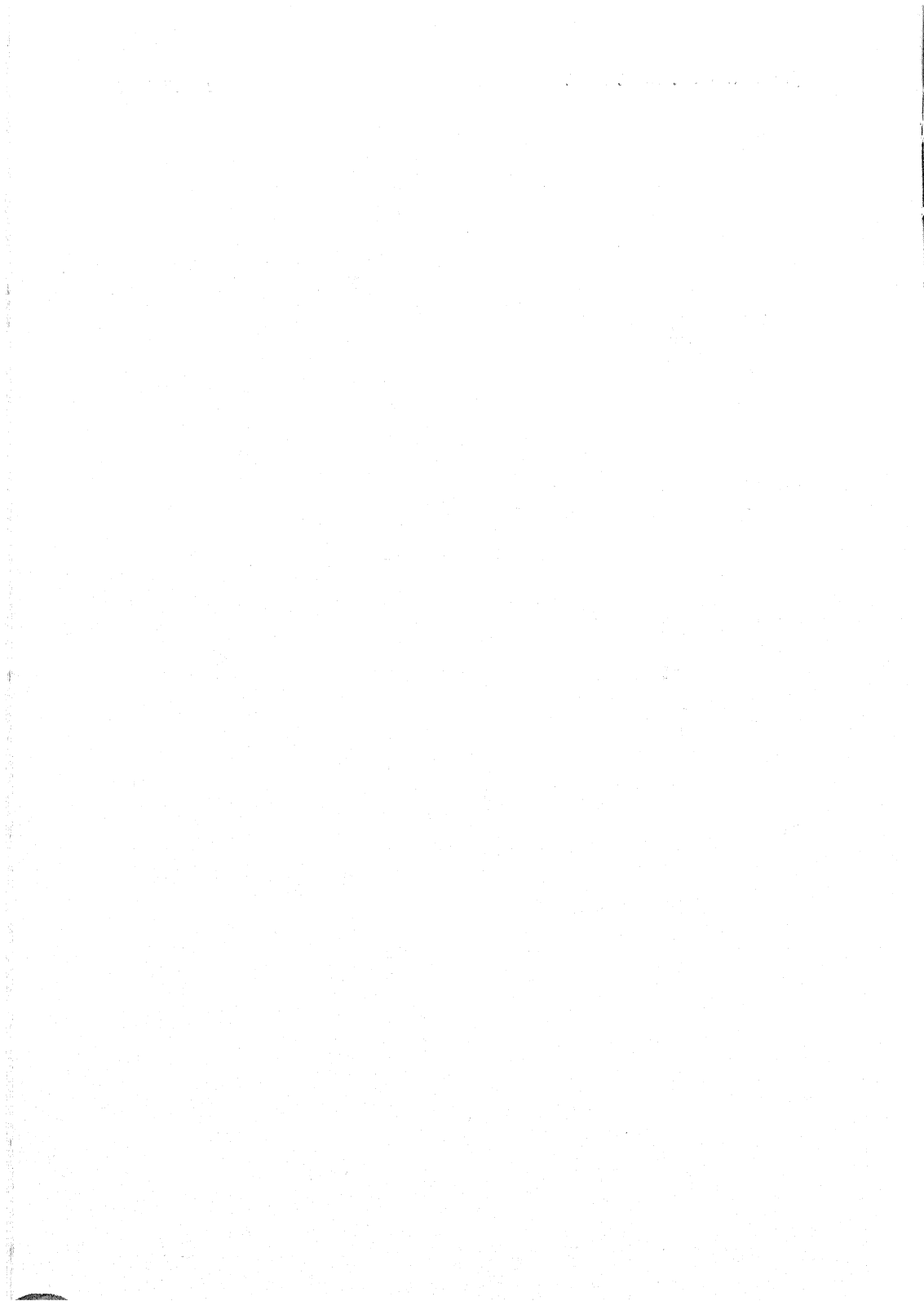
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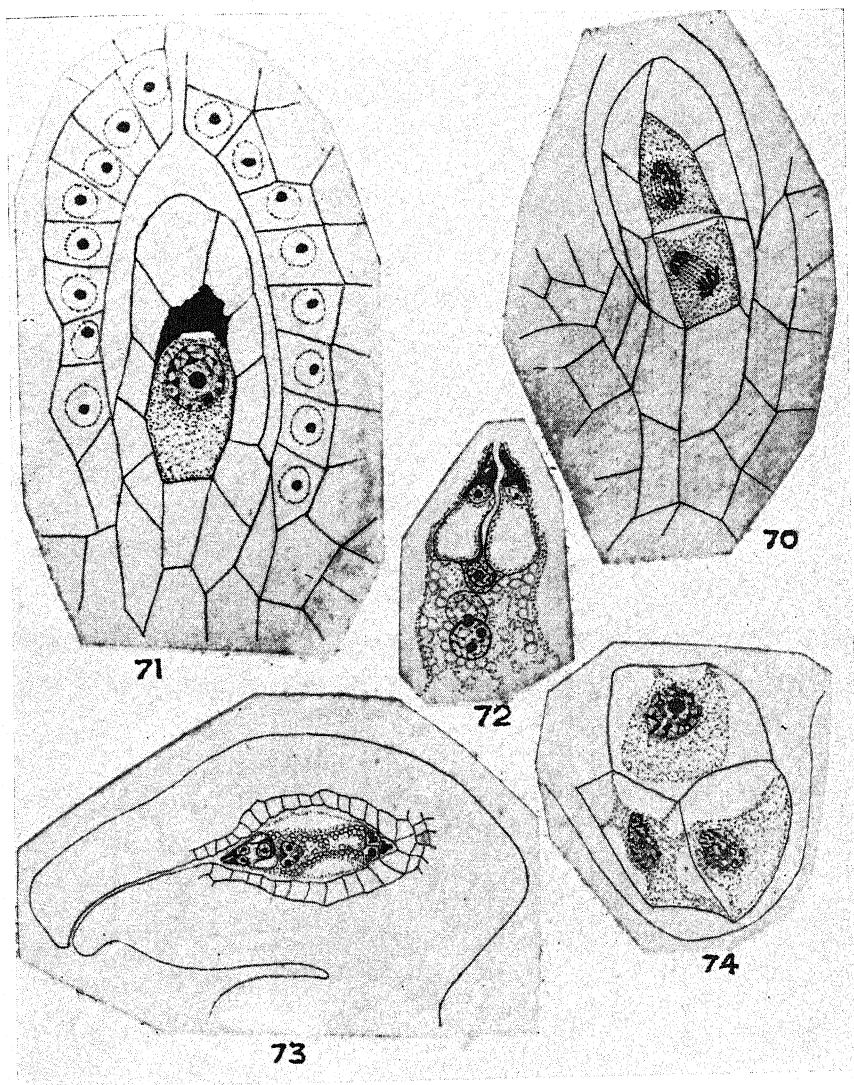


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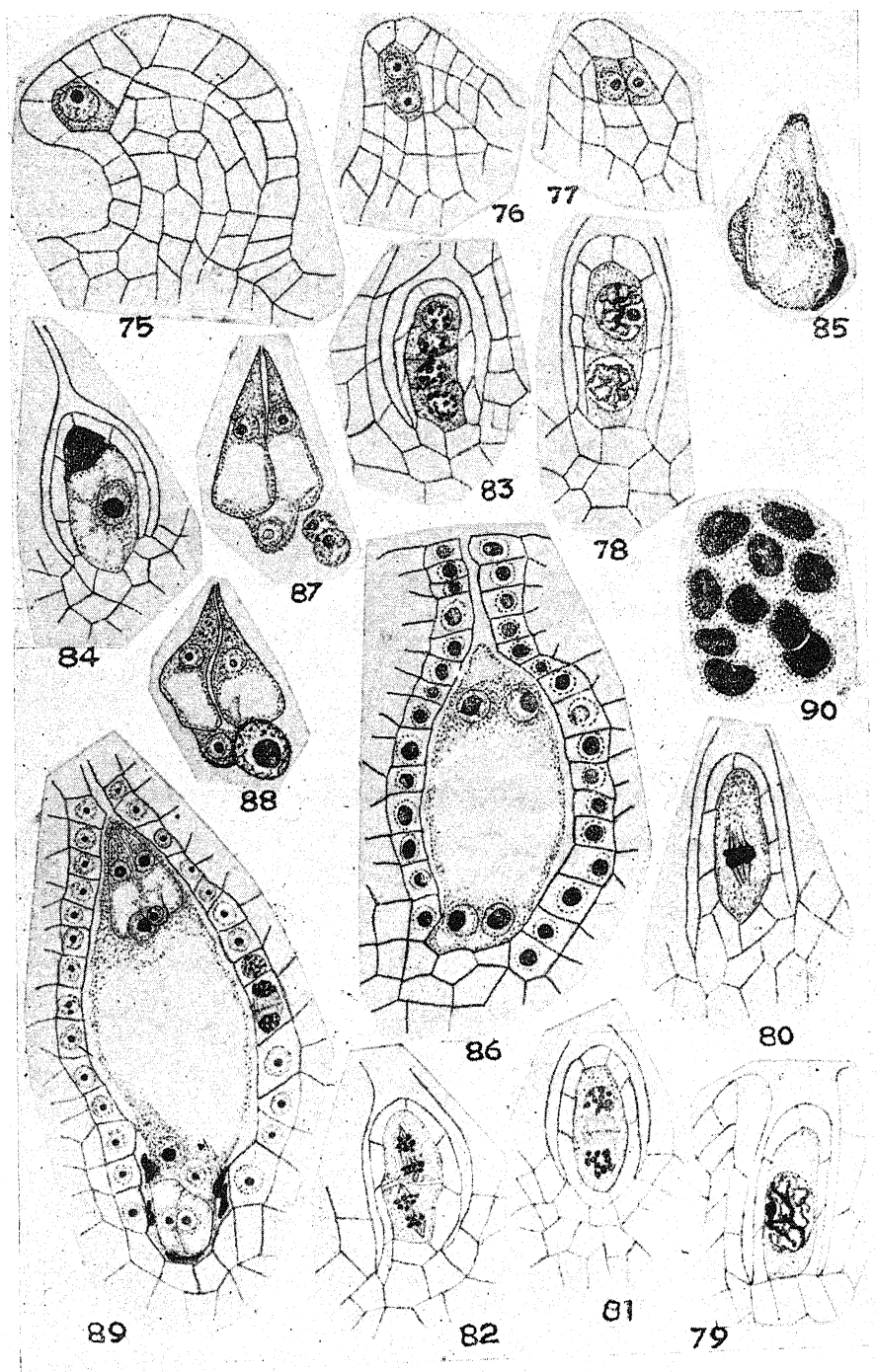




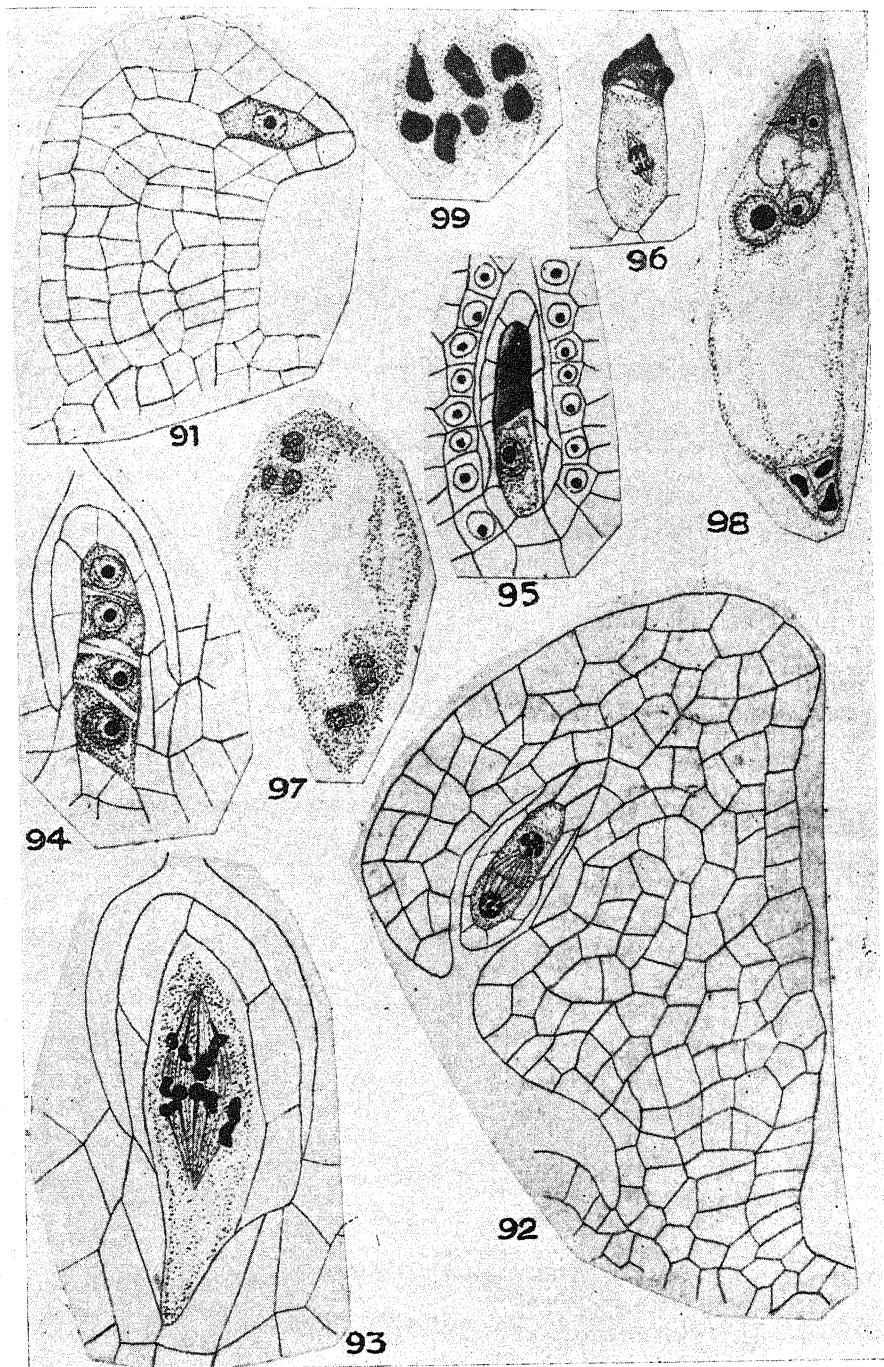


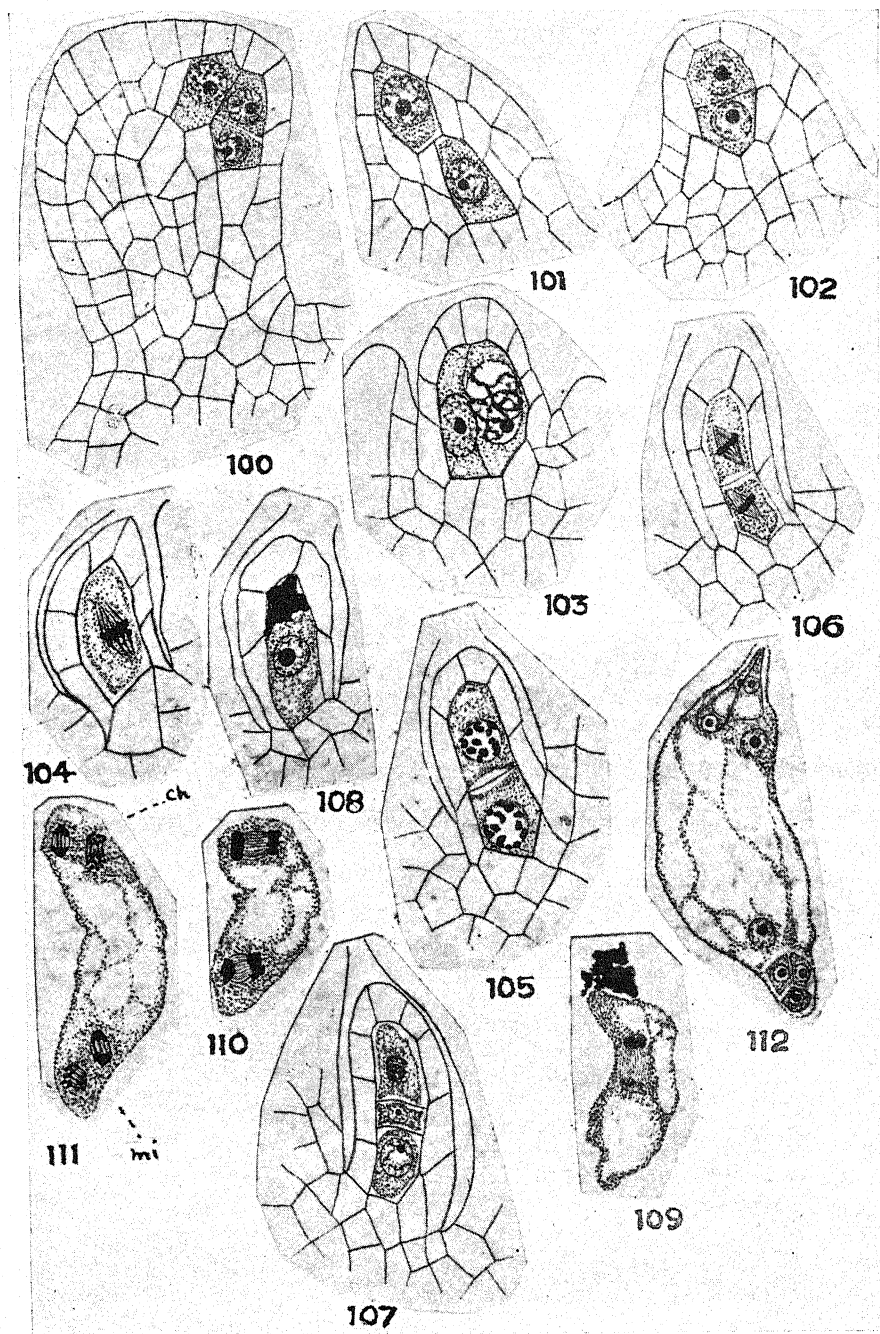


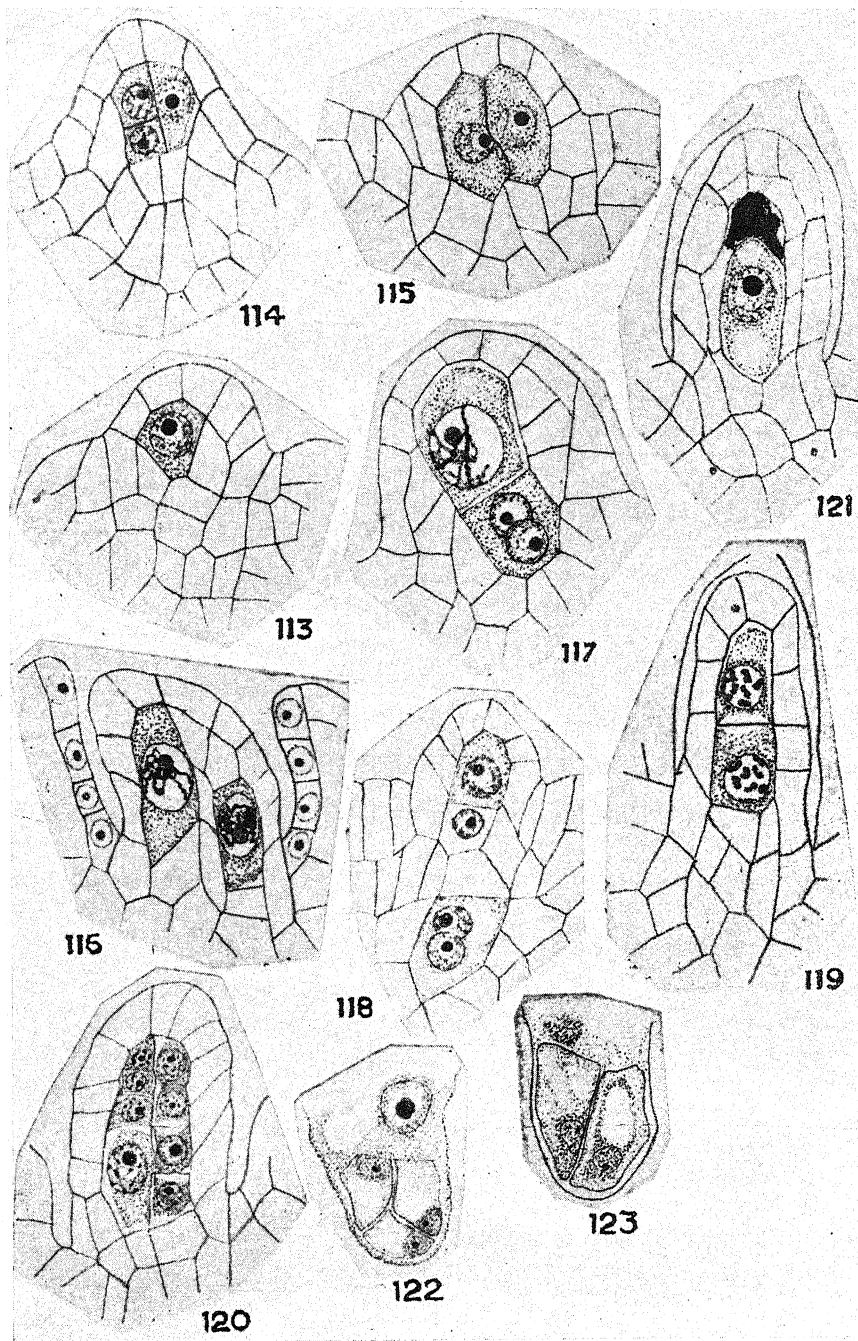


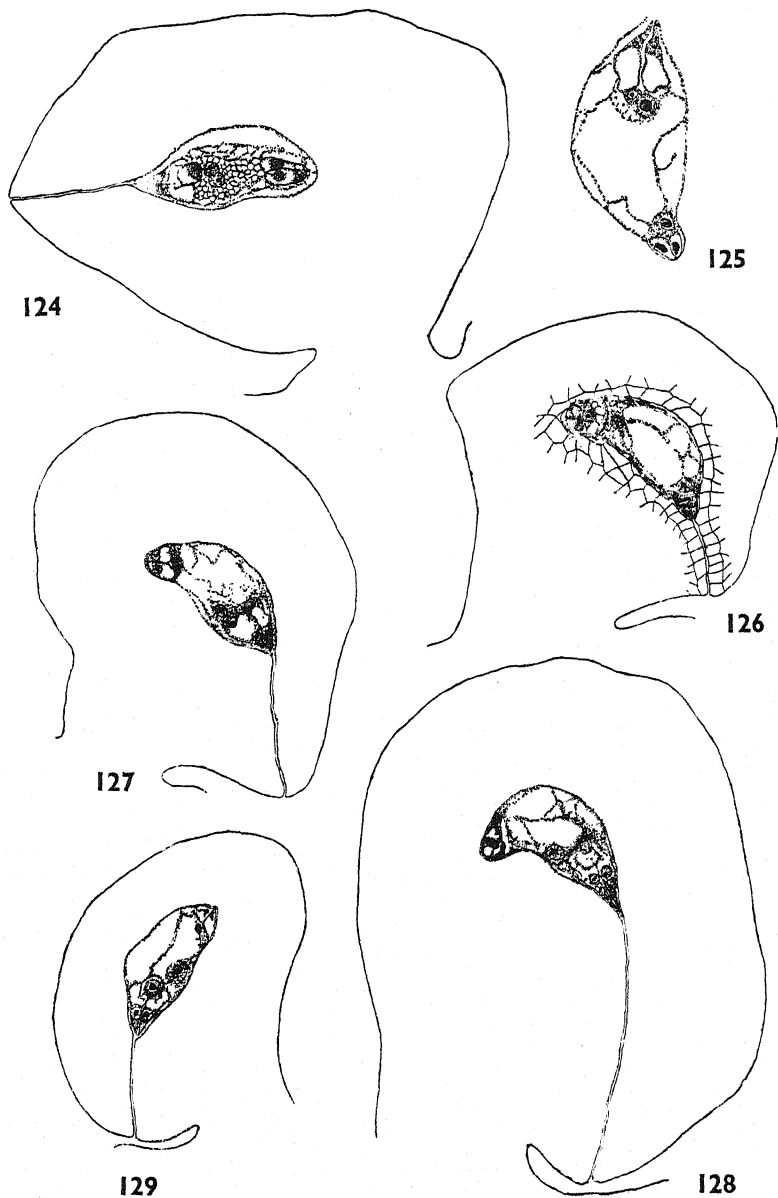




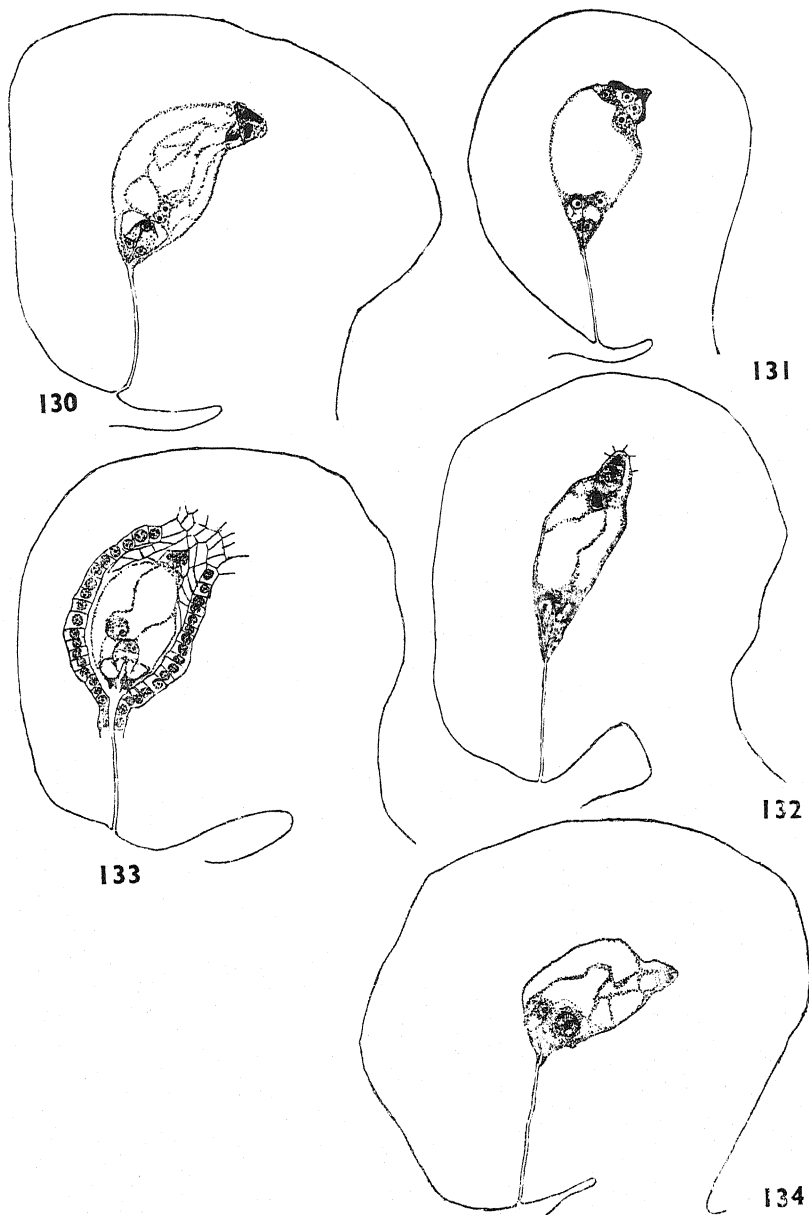


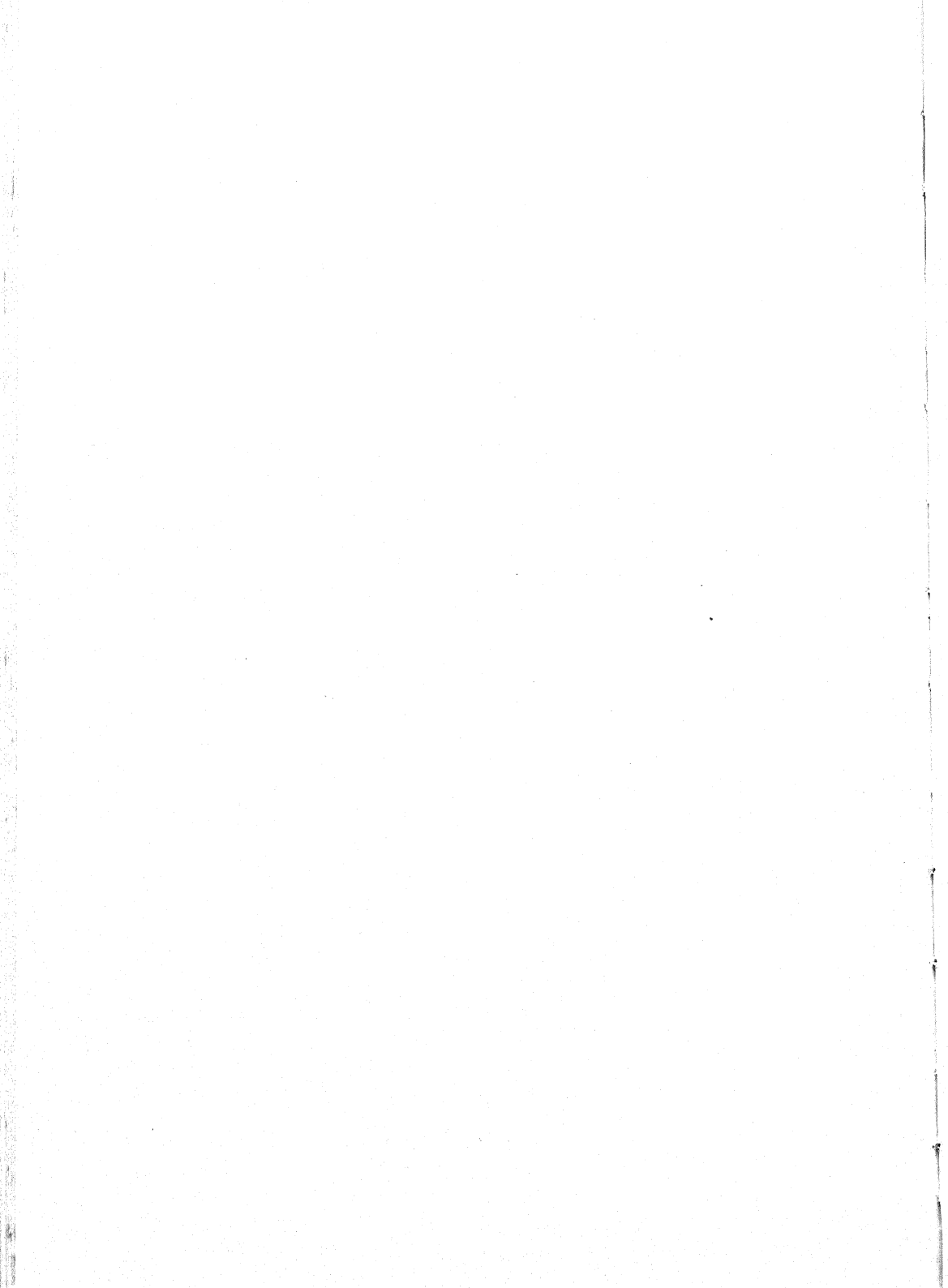




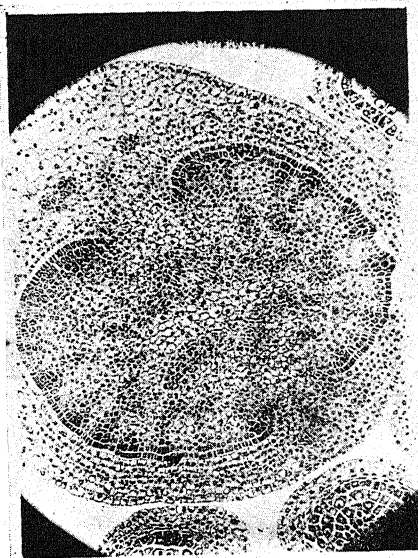








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OBSERVATIONS ON THE SOMATIC CHROMOSOMES OF *URGINEA INDICA* KUNTH

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Received for publication on 6th February 1934.

Introduction

The present paper is a report of observations on mitosis in *Urginea indica*, a common bulbous plant of the east coast of South India. The available literature showed that beyond what has been done by Taylor (16, 17, 18 and 19), Fraser and Snell (5) and Newton (11 and 12) information is meagre as to the number and morphology of the chromosomes of common Liliaceous genera. The papers of Navashin, Sakamura and Bélár have not been available. Very little cytological work has been done on the common plants of South India; a beginning was made on *Allium sativum* (13). Darlington's (1) paper on the chromosomes of the Scillæ deals chiefly with the genus *Hyacinthus*. Schurhoff (14) does not give the chromosome number for any species of this genus. Gaiser (5a) gives the diploid chromosome number of *Urginea maritima* as twenty. It is the intention of the writer to make a comparative study of the chromosome morphology of *Scilla indica* and *Urginea indica*, two closely allied plants of the Scillæ and occurring commonly in South India, and find out how far these two genera are cytologically distinct.

Material and Method

Bulbs were grown in fine sand and after a few days' growth the root apices were fixed. Some of the thicker root tips were sliced longitudinally before fixation. A variety of fixatives was used,—the various modifications of Flemming's fluid, chrome-acetic-formalin of Karpechenko and Langlet (7) and Merkel's fluid. The fixative used by Skovsted (15) for his cytological investigations on cotton, was also tried with considerable success. The best results were obtained with Merkel's fluid and Skovsted's fixative; the former brought out the structure of the chromosomes at different stages most clearly. Sections were cut at a thickness ranging from 6 microns to 30 microns so as to include entire nuclei,

a fact which is important in working out the chromosome complement of selected cells. For staining, both iron alum hæmatoxylin and iodine gentian violet were used. The technique that was found most suitable was that of La Cour (9) involving the use of chromic acid as mordant after gentian violet. With hæmatoxylin, saturated aqueous solution of picric acid was employed for differential destaining after mordanting (10). Gentian violet is much better, especially for prophase stages and for thick sections because of its transparency. The figures were drawn at table level with the aid of an Abbe camera lucida, employing Leitz oil immersion N. A. 1.30 ($\times 100$), and ocular $\times 15$ giving the figures an approximate magnification of 3,500.

Observations

The resting nucleus has a finely granular appearance, and has generally one nucleolus, which is always surrounded by a halo (Fig. 1). The granulation becomes more marked at the commencement of the prophase and it is only then that the threads can be recognised, forming a reticulum (Fig. 2). At a somewhat later stage, the duality of the threads can be made out, each being composed of two chromatids. The threads in the commencement have a coarse non-uniform granular appearance, and are made up of a series of chromomeres of different sizes connected by a less deeply stained thread (Fig. 3). As the threads increase in thickness, the spiral nature of the chromatids could be made out (Figs. 4 and 6). Similar observations on the spiral structure of the prophase chromosomes have been recorded by Newton in *Galtonia* (11), and Kaufmann in *Tradescantia* (8). The chromatids of each chromosome are twisted round one another (Fig. 6); after further linear contraction the spiral structure disappears (Figs. 7, 8 and 9). Even at late prophase, the terminal lobes and constrictions which are so prominent in metaphase and anaphase, can be easily identified (Figs. 6 and 7). By early metaphase the chromatids become much thicker and uniformly cylindrical in form and are held together most closely at the constrictions, which are all of the closely sub-



TEXT FIG. 1

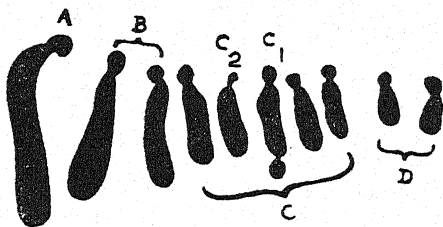
Early metaphase chromosome

terminal type (Figs. 10 and 11. Text Fig. 1). In full metaphase, however, the limbs of the chromosomes come closer together throughout their length (Fig. 12). The morphology of the chromosomes was studied in a large number of full metaphase plates from both lateral and polar views. Chromosomes of the same type differ very much in their length at metaphase and anaphase in the same division

due to different degrees of contraction. No attempt has, therefore, been made to measure the lengths of the different types of chromosomes in a complement. The lengths of the respective types, as shown, represent comparative rather than their absolute size.

The Chromosome Complement

The diploid chromosome number is twenty. All the chromosomes are characterised by constrictions of the closely sub-terminal type. The separation of the two sister chromatids in early anaphase begins at the point of the constriction (Fig. 16) and this region moves foremost to the pole and is therefore held to be the point of attachment of the spindle fibre (Newton 12, Taylor 16). The chromosomes tend to lie with the attachment constriction directed towards the centre of the metaphase plate (Figs. 10 and 12). The larger chromosomes take up more or less a peripheral position in the plate, while the smaller ones are regularly aggregated in the middle (Fig. 10). Homologous chromosomes tend to lie side by side. This "somatic pairing" was also found in *Hyacinthus* by Darlington (1), who attributed it to "mutual adjustment in a



TEXT FIG. 2

The chromosome types of *Urginea indica*

restricted space." The chromosomes of *Urginea indica* seem to fall into four classes on the basis of their size as determined in a number of metaphase and anaphase stages (Text Fig. 2).

A. A pair of long chromosomes with a sub-terminal constriction.

B. Two pairs of chromosomes slightly shorter than A, and also with a sub-terminal constriction.

C. Five pairs of chromosomes, intermediate in size between B and D. Of these one pair C_1 shows a second sub-terminal constriction at its distal end which is unrelated to the fibre attachment.

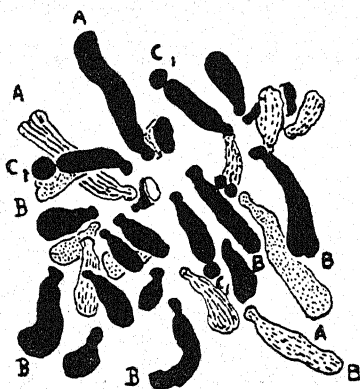
NOTE:—The reproduction of the overlapping of the chromosomes was avoided by slightly moving either the section or the paper sideways between drawing successive chromosomes (Fig. 21).

D. Two pairs of very short chromosomes, with prominent sub-terminal constriction.

This chromosome is clearly seen both in polar and lateral views of metaphase plates. The distal constrictions of this pair are clearly seen directed towards the periphery in figures 13, 14 and 15, while all the other sub-terminal constrictions of the chromosomes are entangled in the centre of the equator. In side views of anaphases, while all the constrictions are directed and bent towards the pole — a fact that points to their being attachment constrictions — (Figs. 20 and 21) the secondary constrictions of the pair C_1 lie nearer the equator, a position which can in no way be related to the fibre attachment. Of the remaining four pairs of C chromosomes, in one pair C_2 , the segment beyond the constriction is minute giving the appearance of a terminal constriction with a trabant or satellite.

Triploidy

Some preparations showed thirty chromosomes instead of the usual twenty, in all the cells of a section and all the sections of individual root tips (Fig. 22). That this is a case of triploidy is shown by the occurrence of the different types of chromosomes of the complement in sets of threes instead of in pairs as in the diploid. This could not, however, be made out with reference to all the chromosomes of the complement owing to the large number. But three of each sort could be traced with reference to a few prominent types. Thus in the side views of anaphases (Fig. 23) and in



TEXT FIG. 3

Metaphase plate of the triploid

polar views of metaphase plates (Text-fig. 3), the three chromosomes with two constrictions each (type C_1) could be readily recognised as also the A and B type chromosomes. It is not known

whether the triploid individual differed in any way morphologically from the diploid, nor if these triploids are hybrids or whether this occurrence of triploidy is due to any aberration of the kind recorded in *Hyacinthus* by Darlington (2) or in *Tulipa* by Newton (12). Triploids are reported to occur commonly in plants "with various kinds of natural vegetative propagation" (3), and their occurrence in the closely allied genera of *Hyacinthus* and *Tulipa* makes it highly probable that triploids are common in *Urginea* also.

Nucleolar Behaviour

There is usually only one nucleolus in the resting stage of the nucleus (Fig. 1). It seems to break up into three or four during the prophase (Fig. 2) presumably by a process of budding (Fig. 5). Circular vacuolar spaces were always recognisable inside the nucleoli while the "halo" around them was also found to be a characteristic of constant occurrence. By the end of prophase the fragmented bits disappear. Occasionally these are seen extruded into the cytoplasm where they disintegrate and probably contribute to the cytoplasmic contents of the cell. Frequently, however, the nucleolus persists up to the full metaphase stage (Fig. 14). In such cases the fragmentation of the nucleolus prior to disintegration which seems to be the characteristic behaviour of this species, does not seem to take place. It is not likely that the nucleolus is in the nature of a store house of the chromatic material contributing to the developing chromosomes. No actual connection between the developing spindles and the nucleoli as recorded by workers like Zirkle (20), could be made out. Nor does the fragmentation of the nucleolus prior to disintegration fit in with such an assumption. Its extrusion into the cytoplasm, though occasional, and its persistence up to the late metaphase stand definitely against such a theory.

Summary and Conclusions

1. The diploid chromosome number of *Urginea indica* is twenty.
2. The complement is resolved into four types which exhibit a tendency for pairing. The smaller chromosomes are aggregated in the centre while the larger ones occupy the periphery of the plate. All of them have constrictions of the closely sub-terminal type. They are attachment constrictions. C₁ has a secondary constriction at its distal end which is unrelated to the point of attachment. C₂ has the segment beyond the constriction reduced to a minute structure, resembling a trabant or a satellite.
3. Triploidy has been noted. The different types of chromosomes occur in sets of threes.
4. The characteristic nucleolar behaviour of this species seems to be fragmentation prior to disintegration. This, together

with its frequent persistence up to late metaphase and occasional extrusion into the cytoplasm, is taken to indicate the absence of any relation between the nucleolus and the chromosomes.

5. The prophase threads are double, being composed of two intertwining chromatids, which themselves have a spiral structure. The metaphasic chromosomes are also double, made up of two cylindrical chromatids, the ends of which frequently present a hollow appearance (Fig. II), a possible fixation result (4). The "daughter chromosomes" derived after anaphasic separation are therefore single cylindrical structures (Figs. 18 and 19), and there seems to be no evidence to support the chromonemata theory of Hedayetullah (6) and Kaufmann (8), which endows the anaphasic chromosome with a dual structure and the metaphasic one with a quadripartite structure. It is believed that the longitudinal splitting of the chromatic thread occurs either in the resting stage or very early in prophase.

In conclusion, the writer wishes to express his indebtedness to Doctors Erlanson and Janaki Ammal for kindly reading through the paper and for many a valuable suggestion and criticism.

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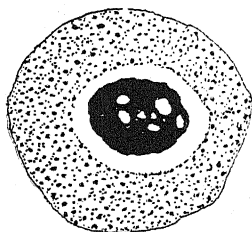
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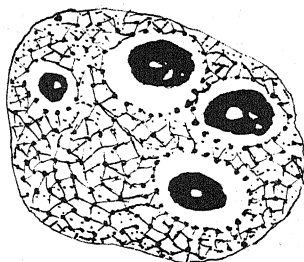
Explanation of Plates 1, 2 and 3

- Fig. 1. A resting nucleus with a single nucleolus. The vacuolar spaces within the nucleolus are visible as also the granular appearance.
- Fig. 2. Early prophase. The threads are evident. The single nucleolus has divided into four bits.
- Fig. 3. Chromomere stage. Duality of threads is seen.
- Fig. 4. Some of the thin threads of the early spireme stage. Their duality and the spiral nature of the chromatids distinctly seen.
- Fig. 5. Spireme stage. The "budding" of the nucleolus also shown.
- Fig. 6. Early spireme when the constrictions can be made out as discontinuous spots at the ends of chromatids.
- Fig. 7. A slightly later stage when the chromatids have become more thickened and the constrictions are seen to occur at the end of each element of the pair.
- Figs. 8 and 9. Late prophase when the nucleoli have disappeared, as also the spiral structure.

- Fig. 10. Early metaphase chromosomes in which the limbs are separate except at the constriction region.
- Fig. 11. Do. The ends of the chromatids present a hollow appearance.
- Fig. 12. Late metaphase, polar view.
- Fig. 13. Metaphase plate showing the two chromosomes with the secondary constrictions at their distal ends (C_1)
- Figs. 14 and 15. Metaphase side views to show the C_1 chromosomes. In 14, the persistent nucleolus can be seen.
- Fig. 16. Anaphasic separation beginning at the region of the constriction.
- Fig. 17. Anaphase polar view.
- Figs. 18 and 19.—Anaphase chromosomes from partially faded preparations to show their cylindrical nature now seen to be hollow.
- Fig. 20. Some anaphase chromosomes showing the constrictions being bent and directed towards the pole. One of the C_1 chromosome can be recognized.
- Fig. 21. Anaphase, lateral view drawn to show the chromosomes separate from one another.
- Fig. 22. Metaphase plate, triploid.
- Fig. 23. Anaphase, lateral view, triploid. A, B and C types can be seen in sets of threes.



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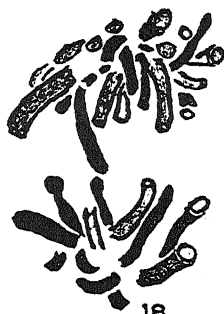


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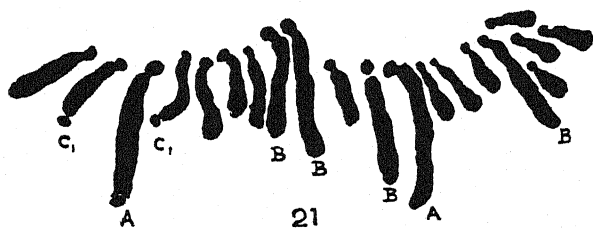
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23

RESPIRATION OF THE ROOTS AND LEAVES OF THE RICE PLANT (*ORYZA SATIVA* L.)

BY

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Received for publication on 21st February 1934.

Different aspects of the physiology of the rice plant, Columbia variety No. 42, are being studied in this laboratory, and it was therefore considered necessary to study the rate of respiration of the roots and leaves of the plant at different stages of growth. The rice plant is semi-aquatic and it would be interesting to measure the rate of respiration of the roots which live in water for the major part of their life. Similarly the measurement of the respiratory activity of the leaves of a plant belonging to the Gramineæ will be of interest as most of the observations of the respiratory rate of leaves include members of other families, especially of dicotyledonous plants.

Smith (1924) has measured the rate of respiration of rice seedlings in her study of the action of anæsthetics on their respiratory activity but the actual amount of carbon dioxide evolved by the seedlings is not stated and the results are given in percentage increase or decrease of CO₂ output.

The respiratory activity of the leaves of Cherry Laurel is determined by Matthaei (1904) and of the leaves of *Helianthus* by Kidd, West and Briggs (1921). In India the respiratory activity of the leaves of tropical plants is determined by Inamdar and Varadpande (1929), Inamdar and Singh (1928), and Ranjan (1925). These workers have studied by means of injection methods the action of various sugars on the respiratory activity with a view to determine the nature of the sugar that is used up in photosynthesis. Their results will be of interest in comparing the respiratory activity of the leaves of the rice plant with those of the leaves in their control experiments.

Dastur and Chinoy (1932) have measured the rate of respiration of the leaves of the rice plant in their experiments on the photosynthetic activities of the leaves in order to obtain true values of the carbon dioxide assimilation of leaves, but their observations are few and are taken towards the evening and do not include the successive hourly readings of the respiratory rates.

The CO₂ output of rice seedlings of different ages has also been estimated by Dastur and Desai under aerobic and anaerobic conditions.

In this investigation the respiration of roots per unit dry weight is measured by a specially fitted apparatus at a temperature of 30°C. An air current deprived of its carbon dioxide is aspirated through a glass chamber in which the respiratory material is kept in a thermostat. The chamber consists of a cylindrical jar with a lid carrying the inlet and outlet gas tubes and a thermometer. The jar and the lid are painted black from the outside. The air is bubbled through very rapidly in order to avoid any accumulation of CO₂ in the chamber which would interfere with the normal respiration of the roots. The air is deprived of its carbon dioxide by passing through sodalime tubes and KOH in towers. The temperature inside the chamber is kept constant at 30°C. by a thermoregulator (toluene mercury) in the bath outside.

The chief problem that required careful attention was the estimation of the carbon dioxide in the air leaving the chamber. Since a very rapid current of air amounting to 100 litres per hour as measured in the gas meter, is passed, the ordinary method of Petten-koffer's tubes containing a barium hydroxide solution of known strength would not be of use as much of the carbon dioxide would remain unabsorbed. Reiset Towers, as used by Kidd, West and Briggs (1921), were employed for absorbing CO₂ by NaOH solutions. A wide mouthed bottle with three holes—two holding the two arms of a Y-tube and serving as an inlet tube and the third holding the outlet tube—was put at the end in order to ensure complete absorption of the CO₂ leaving the chamber. The solution used was 0.2N NaOH.

As the process of preparing carbonate free solution of NaOH proved a very lengthy one, it was given up as it was not found necessary. NaOH solution was prepared by taking a known weight of NaOH sticks (Merck) in a litre of water. The solution was stocked in a big jar which was connected with a burette at the lower end by a glass tube. It was adopted to avoid the absorption of CO₂ from the air.

NaOH sticks contain a certain amount of carbonate and its quantity was determined by titrations with standard HCl. Thus the carbonate already present in the standard solution is determined. This determination was repeated every time a fresh solution was stocked in the jar.

The roots of a bunch of rice plants transplanted about the end of July were taken for each experiment. The plants were carefully uprooted without causing injury and thoroughly washed in water to remove the adhering mud and then placed in the chamber. The air current was drawn for one hour without connecting the chamber

with the absorption apparatus. As the readings had to be taken at almost the room temperature a longer period was not necessary. The absorption apparatus was then connected with the chamber and a reading after one hour was taken. The experiment lasted for seven hours and the mean of five or six determinations was taken. The air current was not interrupted even when the respiratory chamber was disconnected from the absorption apparatus. The roots after the experiments were dried at 100°C to obtain their dry weight. The amount of carbon dioxide evolved was calculated per 100 grms. dry weight. The respiration of the roots from unmanured beds of rice plants was measured at short intervals of not more than a fortnight. The results show a very slight fall in the rate of respiration at first, followed by a small rise in the month of September. After this there is a steady decrease in respiration which is maintained till the end of the season.

The next year 1933 the measurement of respiration was again made to confirm the results of the previous year. The following Table I gives the respiration of the roots of the rice plant in 1932 and 1933:--

TABLE I
Respiration of the roots of the rice plant during
the seasons 1932 and 1933

Date.				CO ₂ in mgs. per 100 grms. dry wt. 1932	CO ₂ in mgs. per 100 grms. dry wt. 1933
August	9	201	196.4
August	20	165	184
August	27	168.2	..
August	30	176
September	10	182	..
September	14	153	190.5
September	27	182
September	30	147	..
October	11	124	119
October	20	145	113.4
November	2	93	82.1

The graphs showing the respiratory rate of the roots in the two years are shown in Fig. 1.

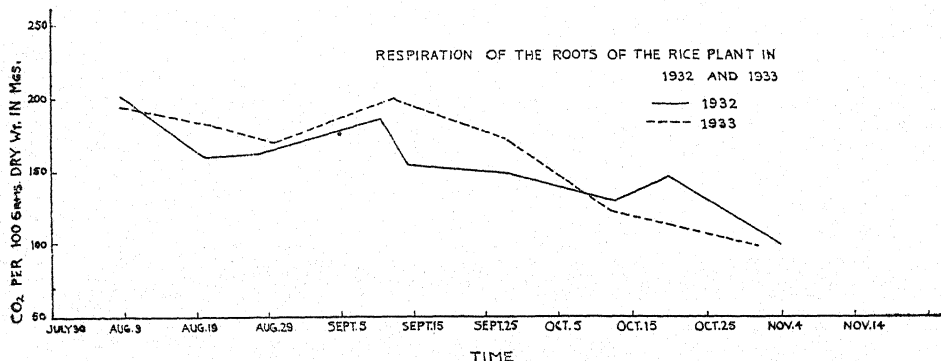


FIG. 1

On comparing the results of the two years it is found that they are strikingly similar. There is a slight decrease in respiration followed by a slight increase and then there is a fall till the end of the season. From Fig. 1 it can be seen at a glance that the graphs showing the rates of respiration of the roots during 1932 and 1933 are very near each other and run almost on parallel lines. The fall in the rate of respiration of the roots towards the end of the season indicates probably that the roots failed to function owing to internal causes though they were continuously watered. This is what should be expected because towards the end of the season the roots near the termination of their functional activities.

In a few cases the respiration of the roots of plants which were previously manured was measured and it was found that the manured plants showed a higher rate of respiration than the unmanured ones. The results are not confirmed. Hence they are not included here.

Respiration of Leaves

The respiration of the leaves was measured along with the respiration of the roots. In the beginning just after transplantation, all the leaves from a bunch of rice seedlings were taken for measurement, but later on it was found inconvenient to take all the leaves for each measurement of respiration as the respiratory chamber was found rather small for the purpose; so a few leaves were taken each time. The leaves were selected so as to include the leaves of different ages to get an average reading for the respiration of the leaves of the whole plant. As in the case of the roots, it is found that there is a decrease in the rate of respiration of the leaves followed by a slight increase and then there is a fall; but the fall is

not so gradual and at the end of the season the respiration, though not as vigorous as at the beginning, is still appreciable.

Similar experiments were done the following year. The respiration is found again to be high in the beginning after transplantation and then there is a fall. It is practically constant in September when there is a slight rise and again a gradual decrease in respiration is observed. Table II gives the respiration of the leaves in 1932 and 1933.

TABLE II
Respiration of the leaves of the Rice plant during
the seasons 1932 and 1933

Date.			CO ₂ in mgs. per 100 grms. dry wt. 1932	CO ₂ in mgs. per 100 grms. dry wt. 1933
August	11	..	319	402
August	23	..	278	312
August	30	290
September	2	..	264	..
September	9	..	286	..
September	12	232
September	20	..	295	289
September	28	293
October	1	..	197	..
October	14	190
October	17	..	187	..
October	21	175
November	6	..	163	178.3

The graphs showing the rates of respiration during the two seasons are shown in Fig. 2.

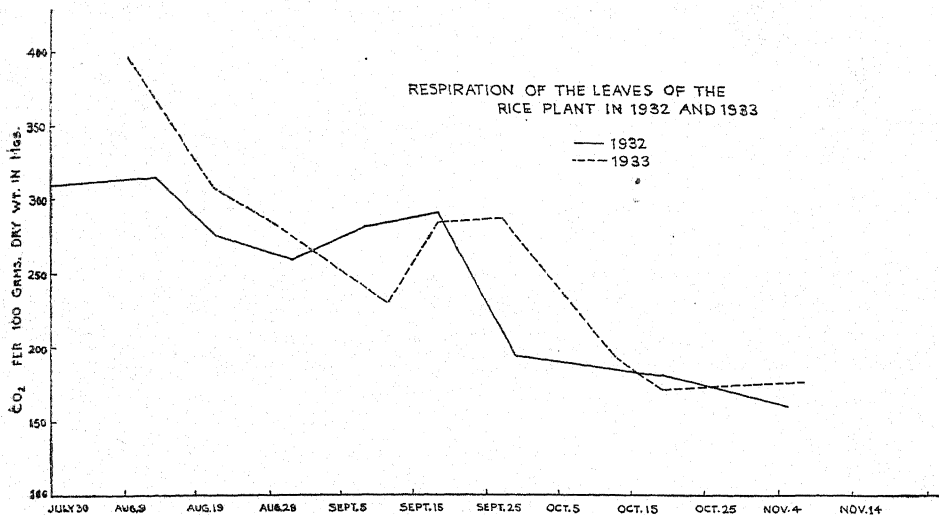


FIG. 2

It is found that the fall in the case of the leaves at the end of the season is not so gradual as in the case of the roots. The respiratory activity in all cases is found to be high in the beginning just after transplantation. In August and September the rate of respiration is found to be almost constant.

Conclusions

The rate of respiration of the roots fluctuates between 82 and 200 mgs. per hour per 100 grams of dry weight while that of the leaves is nearly double that of the roots, fluctuating between 163 and 400 mgs. per hour per 100 grams of the dry weight of the leaves. The rate of respiration of the leaves of the rice plant is not low as compared to the rates of respiration of the leaves of other plants like the Cherry Laurel or *Helianthus* determined by other workers. For Cherry Laurel leaves as determined by Matthaei (1904) it was 0.8 of a milligram for 2 grams of leaves. So even making allowance for the dry weight of the leaves, the rate of respiration is lower than that of the rice leaves. For *Helianthus* as determined by Kidd, West and Briggs (1921) the rate of respiration fluctuates between one and three milligrams and for *Artocarpus integrifolia*, as determined by Inamdar and Varadpande (1929) the rates of respiration vary from 1 to 3 milligrams per hour per unit dry weight. The higher rates of respiration of the leaves of the rice plant may be due to the use of Reiset Towers

with an additional bottle containing NaOH solution by means of which it is possible to pass a very rapid current of air amounting to 100 litres per hour. This is not possible when Petten-koffer's tubes containing baryta water are used.

There is a marked fall in the rate of respiration soon after transplantation and the maximum is reached somewhere in the middle of September. It is remarkable that there is no increased rate of respiration of the leaves in the 1st or 2nd week of October when the photosynthetic activity of the leaves reach the second maximum point. The rate of respiration of the leaves shows on the contrary a steep fall soon after the maximum is reached in September.

Summary

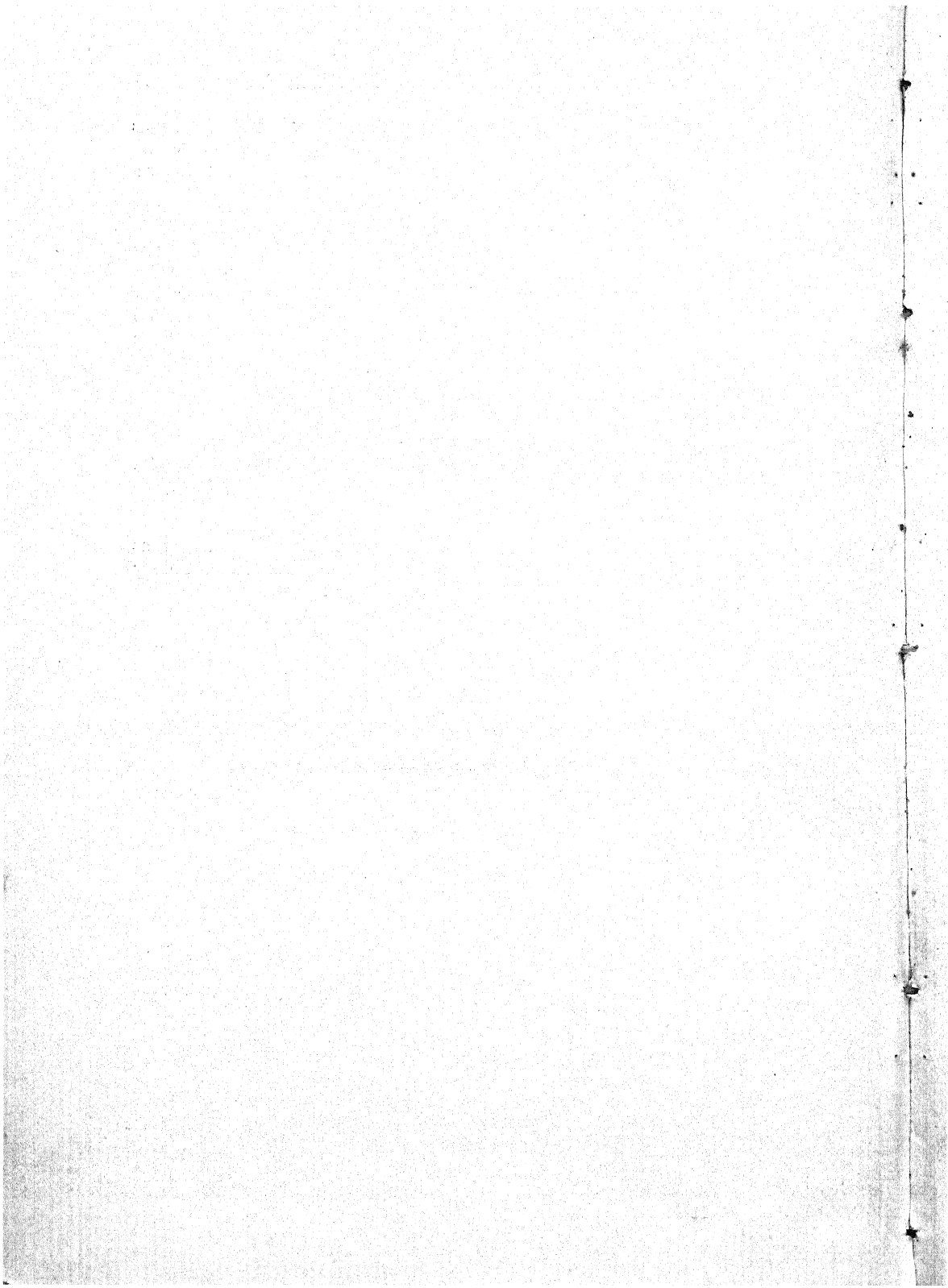
In the case of the roots the amount of CO₂ given out varies between 82 and 200 milligrams and in that of the leaves between 163 and 400 milligrams per 100 grams dry weight.

The rate of respiration of the leaves of Rice is not low as compared with the leaves of other plants like *Helianthus* or *Cherry Laurel*.

I wish here to express my deep sense of gratitude to Prof. R. H. Dastur, the Head of the Botany Department, Royal Institute of Science, for the interest he has always evinced in this work and for his help and guidance during the course of this investigation.

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OBSERVATIONS ON THE ARTIFICIAL GERMINATION OF *CYATHODIUM* SPORES

BY

N. K. TIWARY, M. SC.

Received for publication on 16th January 1935.

The germinating spores of *Cyathodium* were discovered and described for the first time by the writer^{a, b}, a few years back. The description was based on the observations made on the spores found germinating under natural conditions. Some gaps necessarily remained. In order to get information about these and other points, and in general, to institute a comparative experimental study under various external conditions — a phase of study which has been sadly neglected with respect to the spores of the Bryophyta as a whole — an attempt was made to make artificial cultures in the laboratory. A preliminary account of this was reported in 1930.^c Those observations have since been amplified.

The original plan was to make cultures in different media. This, however, could not be accomplished. The following account is based on the observations so far made on "hanging drop" cultures only, kept under bell jars under ordinary laboratory conditions of light and temperature.

As already reported (*loc. cit.*), the spores require an obligatory period of rest before they can germinate. This has since been also confirmed by Khanna,^d working on the spores of *Cyathodium Kashyapii*, in Burma. No observations are available regarding the minimum and the maximum limits of the resting period — before and after which the spores do not germinate. In the case under report, the freshly gathered spores, and spores a few weeks old, did not germinate. This they readily did after about nine months' storage. As regards the upper limit of viability, the specimens collected in 1930 failed to germinate when an attempt was made last October (1934), after almost exactly four years' storage, although the spores looked apparently quite fresh and healthy.

The first indication of germination is an evident increase in size by swelling, which is recognisable when the dry spore is

a. Tiwary, N. K. 1929, p. 1001.

b. Tiwary, N. K. 1929, pp. 19-143.

c. Tiwary, N. K. 1930, p. 288.

d. Khanna, L. P. 1932, pp. 99-101.

brought in contact with moisture (Plate II, Fig. 1-b.) This increase, however, does not necessarily imply that germination would result in every case. Some spores, in fact, after undergoing this preliminary increase, may not germinate at all. Even non-viable specimens, such as those sown after four years of storage, increased in volume after wetting. It thus appears to me merely a mechanical phenomenon, such as accompanies the wetting of dry wrinkled objects.

In the observations here recorded, no other change could be noticed until the third day from the starting of the cultures. After this lapse of time, the spore-coat, which is more or less opaque, or only slightly translucent, begins to get more and more transparent. The contents which could only be faintly made out, at first, as a darker mass in the centre, showed up as a distinct big nucleus, suspended by thick strands of protoplasm (Plate II, Fig. 7). On this day, too, one spore, out of a total of 25, began to put forth the germ-tube. By the sixth day almost 95% of the specimens germinated, producing fairly long germ-tubes.

In germination, the spore-coat does not show any special mode of rupture (Plate I, Figs. 1 & 2. Plate II, Figs. 2-a, 2-b, 3, 3-a). It is simply stretched by the expanding endospore, in the same way as mentioned by Campbell,^e for the Marchantiales and the Jungermanniales, and the latter then grows out through one or two places according to circumstances. There is evidence, as will be clear from the subsequent account, of the existence of "fixed" potential germ-pores, although structurally the exine does not seem to be differentiated.^f

In the majority of the cases here recorded, the endospore developed on one side only, producing the germ-tube (Plate I, Figs. 1-3, 6-11, 13, 15. Plate II, Figs. 2-a, 2-b, 3, 3a- 6, 7) though in a few specimens, it developed through two opposite points, the other producing the rhizoid (Plate I, Figs. 4, 10, 12, 14), a condition almost of universal occurrence under natural conditions.

The development of chlorophyll starts before the initiation of the germ-tube. On the latter developing, this is expedited. At first the chloroplasts are fewer and smaller, but as the germ-tube grows into a cellular filament, these increase both in number as well as in size.

In the spores, to which the present study relates, the germ-tube without a single exception, developed into a filamentous structure, consisting of a single row of elongated cells. In no cases was a germ disc formed, such as ultimately develops under

^e. Campbell, D. H. 1913, pp. 66 and 113.

^f. Goebel also mentions (See pp. 676 and 767 in 5 *infra*) the existence of polarity in the spores of *Lejeunia* and *Odontolejeunia*.

natural conditions and produces the thallus. Only in one case (Plate I, Fig. 11) did the end cell of the germ-tube show indication of branching. Such a development of filamentous structure has been usually associated with feeble light intensity *g, h, i, j* and Goebel (*loc. cit.*) specially, frequently refers to this correlation. It may, however, as well be due to excessive moisture, specially as regards the present observations.* This requires further investigation.

The rhizoid formation ensued only in four spores. In two of these cases (Plate I, Figs. 10 and 14) the development did not begin until the eighth day after sowing. In the others, too, it happened quite late. This appears to be certainly related to the excessive moisture conditions of the "moist chamber". When developed they regularly emerged (with rare exceptions) from a point on the exosporium opposite the germ-tube, thus confirming the existence of polarity in the spores, as already observed and described by the writer.^k The existence of such polarity has been called into question by Khanna.^l In view of the present observation, however, it would appear at least necessary to re-examine the position stated by him.

An impression generally prevalent with reference to the existence of the germ-tubes and the rhizoids, is that the former develops on the side exposed to the incident light, and the latter on the side opposite. This is not borne out by the germinating *Cyathodium* spores. As will be seen from the figures, no such connection necessarily exists. In many cases the germ-tubes started developing at various angles to the source of the incident light though often bending later suitably to become parallel to it (cf. Figs. Plate I). An analogous behaviour of the rhizoids is strikingly shown by the only cases where these were developed (Plate I, Figs. 4, 5, 12, 14). In one case, indeed, the rhizoid was still growing more or less facing the incident light (Plate I, Fig. 12.) It appears permissible to draw the conclusion, that this feature is related to the existence of the germ pores, fixed in position on the spore case, though they do not appear to be morphologically indicated. The phototropic behaviour of one of the rhizoids, however, appears mysterious.

A feature of some striking interest relates to the occurrence of certain elongated highly refracting, homogeneous contents of some cells in the germ-tubes, in a number of cases (Plate I, Figs. 8, 9, 10, 12, 15. Plate II, Figs. 5, 5-a, 5-b, 8, 8-a). They

g. Campbell, D. H. *loc. cit.*, p. 20.

Goebel, K. (1915-1918), pp. 759-760, 765, 769.

i. Pande, S. K. 1924, pp. 119-120.

j. Tiwary, N. K., *loc. cit. a, b.*

* Goebel also mentions (See *loc. cit.*, pp. 676 and 767) in the existence of polarity in the spores of *Lejeunea* and *Odontolejeunea*.

k. Tiwary, N. K., *loc. cit. a, b.*

l. Khanna, L. P., *loc. cit.*

were perfectly transparent, and appeared to be, from the mere look, oily in nature. They were generally observed in certain intermediate as well as terminal cells, extending through the common cell wall of the adjacent cells. Further, they were found to alter their position from day to day. Before further observations could be made on these bodies and on other stages of germination, the culture was suddenly destroyed.

It was also incidentally noticed that the chloroplasts occurred in the largest number in the terminal cell. Furthermore, a number of them were observed to become associated in a group, whose position varied from day to day (Plate II, Figs. 5, 5-a, 5-b.)

The operation of the various external conditions on the germinating spores of Bryophyta, as the late Prof. Goebel remarks in his *Organographie*^m has not been satisfactorily worked out. Even the exact nature of the influence of the few factors that have been experimentally studied is but imperfectly understood. Thus this almost unexplored region offers a rich field for the study of experimental morphology. The writer has already collected ripe sporogonia of various liverworts, both from the plains and the hills, with a view to studying the germination of their spores under varying external conditions. The results of these studies will be reported in due course.

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EXPLANATION OF PLATES

All figures have been drawn with the aid of camera lucida and the spores are shown in their natural position in the "moist-chamber."

PLATE I.

(All figures are magnified $\times 290$ diameters).

Figs. 1—3 Spores showing earlier stages of germination.

Figs. 4—5 Two spores in later stages of germination showing the origin and the relation to incident light of the germ-tube and the rhizoids.

^m. Goebel, K., *loc. cit.*, p. 769.

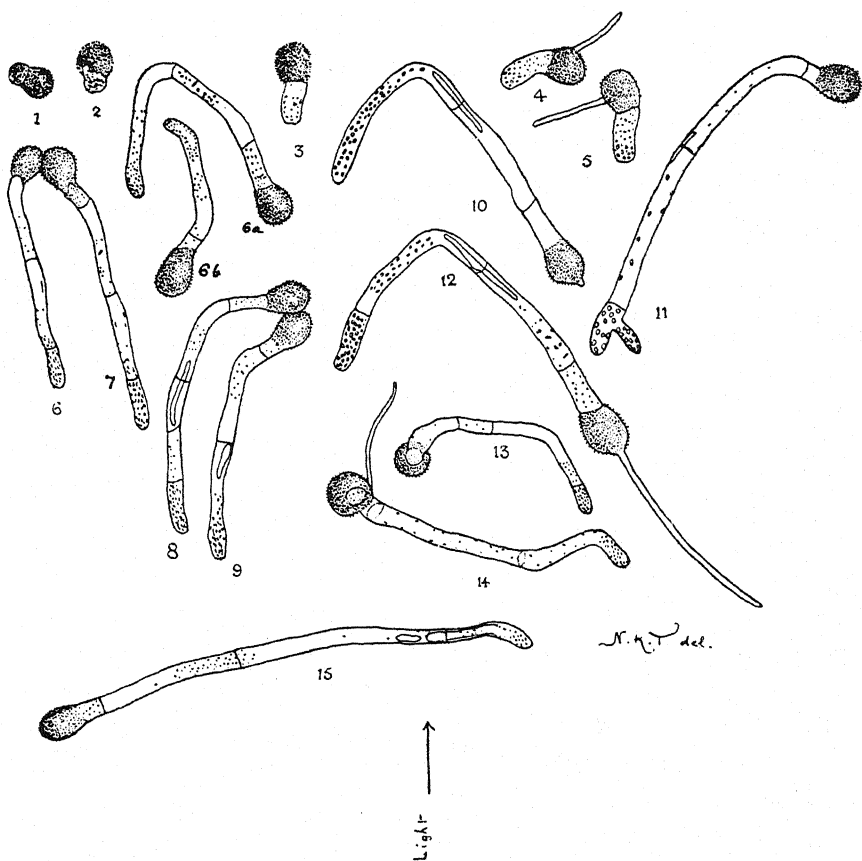
Figs. 6—15 Spores after several days of germination, in their natural position. Rhizoids are developed only in Nos. 12 and 14. No. 11 shows a branched terminal cell. (No. 10 is an earlier stage of No. 12). In Figs. 8, 9, 11, 12 and 15 are shown peculiar elongated "oily" contents in the cells (cf. text). No. 12 shows a rhizoid still growing toward incident light.

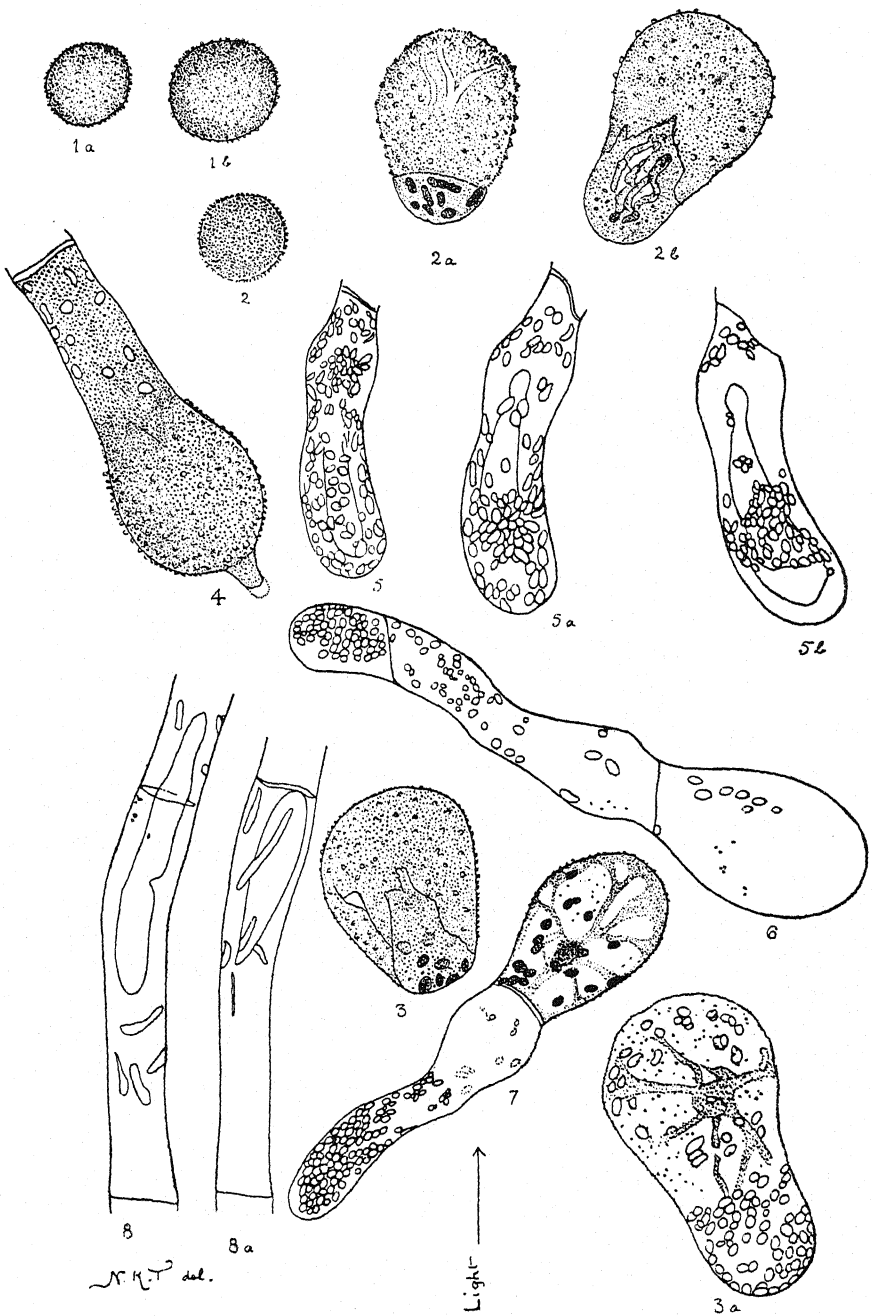
PLATE II.

(All figures are magnified $\times 870$ diameters).

- Figs. 1-a, 2 Spore when dry.
- Fig. 1-b Spore soon after wetting.
- Figs. 2-a, Showing rupturing of the spore-coat during germi-
2-b, 3, 3-a nation. (Fig. 3-a, represents stage of 3 after 24 hours
germination.)
- Fig. 4 Shows the exit of rhizoid at a place opposite that of
the germ-tube.
- Figs. 5, 5-a, Different appearances of the same terminal cell on
5-b three successive days. They also show the peculiar
elongated 'oily' contents (cf. text).
- Figs. 6, 7 Two spores with their germ-tubes growing at different
angles to the incident light. Fig. 7 is an optical section
showing up the contents, through transparent spore-
coat.
- Figs. 8 Shifting position of 'oily contents' (cf. text) in the
8-a. same cell of the filament on successive days.







INHERITANCE OF ROOT COLOUR IN RICE *

BY

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Crop Botanist to the Government of Bombay.

Received for publication on 3rd April 1934.

Introduction

The inheritance of various colour characters has been extensively investigated in rice. There appears, however, no report on the genetics of root colour in rice or any other grain crop. This may be due to the rare occurrence of varieties with coloured roots, and the practical difficulties attendant in the course of such an investigation.

During the monsoon of 1930 the writer, while examining the seedlings of various rice varieties grown at the Rice Breeding Station, Karjat, Kolaba, noted deep red colour on the exposed parts of roots of a Burmese variety. A number of seedlings of this variety were exposed to sunlight in bottles containing water. In a few days bright crimson colour developed on the new roots. The Burmese variety was crossed with an entirely colourless variety to investigate the mode of inheritance of root colour. The results are reported in this paper.

Review of Literature

The inheritance of root colour has been largely investigated in such crops as *Beta vulgaris*, *Brassica Napus esculenta*, *B. rapa esculenta*, and *Raphanus sativus*.

According to Matsuura (1929), Kajanus has established two genes R and G producing anthocyanin pigment in *Beta vulgaris*. The two genes together produce red colour. G alone produces yellow and absence of both or R alone results in white. Later on Lindhard and Iverson (1924) showed that the genes R and G are linked about 36 to 38 per cent., which explained the discrepancies encountered by Kajanus.

In *Brassica Napus esculenta*, Kajanus, according to Matsuura (1929), found two genes P_1 and P_2 for anthocyanin pigment in roots. P_1 causes pale violet colour and is hypostatic to P_2 which produces deep violet pigment. Absence of both results in colourless roots.

Recent researches of Davey (1932) establish two duplicate genes N_1 and N_2 which produce red colour on the skin in *Brassica Napus*. Davey redefines Kajanus' factor P_2 as N_1 .

* Paper read before the Twenty-first Annual Meeting of the Indian Science Congress held at Bombay from January 2nd—7th, 1934.

In radishes Uphof (1924) finds red colour of roots dominant to colourless. In some of his crosses he found monogenic relationship, while in a commercial variety he found red colour due to two duplicate genes, R_1 and R_2 . In his material Uphof could discern pigment in the seedling stage only as the red colour disappears when the roots become fleshy.

Material and Methods

The Burmese variety (No. 355) was crossed during the season of 1930 with *Ratanghose* (No. 257), a dwarf and an entirely green variety originally obtained from the Thana district of the Bombay Presidency. The name of the Burma variety is not known. The two varieties differ greatly in their blooming period, but by utilising late tillers of Ratanghose crosses were obtained. The crossed seeds were sown in the season of 1931 and four successful crosses were obtained. The F_1 plants resembled the male Burma parent. Unfortunately the root colour of the F_1 plants was not noted.

With the commencement of the monsoon of 1932, seeds from one of the F_1 plants were sown in seed beds. When the seedlings were about three weeks old they were uprooted, washed and exposed to sunlight in bottles containing water. The bottles were left in the open during forenoon and late in the afternoon; and removed to shed from 11 a.m. to 3 p.m. to prevent the plants from wilting. After noting the colour of roots the plants were transferred to the field.

The F_2 generation was similarly studied during the season of 1933.

In judging the significance of the observed results from the expected, the method of X^2 has been used (Fisher 1932). The level of significance is drawn at the 0.05 point and appropriate degrees of freedom are taken to obtain the values of P .

Experimental Results

F_1 Generation

As has been stated the root colour of the F_1 plant was not noted. There can be, however, hardly any doubt of its being coloured.

F_2 Generation

In the F_2 generation, out of the 528 plants 299 had coloured roots; while 229 were colourless. The segregation was very close to the expected frequencies on the basis of the digenic ratio of 9 coloured: 7 colourless; the deviation being of only two plants from the theoretical expectation. The plants with coloured-roots will be hereafter referred as "coloured" and those without colour as "colourless". The F_2 data are shown in the following table:—

TABLE I.
Segregation of root colour in the F_2 of ♀ Ratanghose
X ♂ Burma.

F_2 Class	Observed	Expected	X^2/m	P.
Coloured roots	299	297	·0134	
Colourless roots	229	231	·0173	
Total	528	528	$X^2=·0307$	0·90 — 0·80

F_3 Generation

From the two groups of the F_2 , plants were separately taken at random to study the segregation of root colour in F_3 . Plants which had no root colour development in the F_2 bred true in the F_3 generation. Plants that were coloured in the F_2 showed varying breeding behaviour in F_3 . Out of the 19 such F_2 cultures 2 bred true, 9 segregated in the monogenic ratio of 3 coloured : 1 colourless, and 8 segregated in the proportion of 9 coloured : 7 colourless.

The individual behaviour of cultures showing monogenic segregation in F_3 is shown in the following table:—

TABLE II
Cultures showing monogenic segregation of root
colour in the F_3 of ♀ Ratanghose X ♂ Burma.

Culture No.	Observed.		Expected 3 : 1.		Total.	X^2	P.
	Coloured roots.	Colourless roots.	Coloured roots.	Colourless roots.			
39	61	25	64·5	21·5	86	0·759	0·50 — 0·30
86	18	7	18·75	6·25	25	0·120	0·80 — 0·70
100	46	18	48·00	16·00	64	0·333	0·70 — 0·50
267	19	6	18·75	6·25	25	0·013	0·95 — 0·90
333	78	19	72·75	24·25	97	1·515	0·30 — 0·20
338	20	5	18·75	6·25	25	0·333	0·70 — 0·50
546	21	4	18·75	6·25	25	1·080	0·30 — 0·20
548	20	5	18·75	6·25	25	0·333	0·70 — 0·50
571	19	6	18·75	6·25	25	0·013	0·95 — 0·90
Total of all cultures.	302	95	297·75	99·25	397	0·242	0·70 — 0·50

It will be noted from the above table that the agreement between the theoretical and the observed frequencies is very good in all cases. The values of P are well over the level of significance.

The total number of plants from all the nine cultures is 397, out of which 95 have colourless roots and the remaining 302 show coloured roots. The deviation from the expected segregation being only of about 4 plants.

The behaviour of coloured cultures showing digenic segregation of 9 coloured roots; 7 colourless roots is shown in the table below:—

TABLE III
Cultures showing digenic segregation of root colour
in the F_3 of ♀ Ratanghose X ♂ Burma.

Culture No.	Observed.		Expected 9 : 7		Total.	χ^2	P
	Colour- ed roots.	Colour- less roots.	Colour- ed roots.	Colour- less roots.			
6	50.	38	49.50	38.50	88	0.012	0.95 — 0.90
48	53	32	47.80	37.20	85	1.292	0.30 — 0.20
57	50	29	44.44	34.56	79	1.590	0.30 — 0.20
79	41	36	43.31	33.69	77	0.282	0.70 — 0.50
179	14	11	14.06	10.94	25	0.0006	0.99 — 0.98
306	16	13	16.31	12.69	29	0.013	0.95 — 0.90
386	12	8	11.25	8.75	20	0.114	0.80 — 0.70
357	109	84	108.56	84.44	193	0.004	0.95 — 0.90
Total of all cultures	345	251	335.25	260.75	596	0.648	0.50 — 0.30

It will be seen that every culture is showing a good agreement with the expected figures on the basis of a 9:7 ratio. All the values of P are well over the 0.05 point. The total of 596 plants, from all the eight cultures, is divided into 251 plants with colourless roots and 345 with coloured roots; the frequencies deviating by only about 10 plants from the expected segregation.

Genic Interpretation

From the foregoing data it will be clear that we are dealing here with two genes whose co-operation is necessary to produce colour on roots. Absence of either one results in colourless roots. Unpublished analysis of other gene effects in the Ratanghose-Burma cross show that one of the genes is the major gene for anthocyanin pigment which must be present, in addition to other specific genes, to have colour produced in any part of the plant. If this gene is absent the plant is entirely green, irrespective of the presence of other colour-genes. The gene is designated **A**. The other is the specific root-colour gene which is required, in addition to **A**, to produce anthocyanin pigment on the roots. The root-colour gene is termed **R_o**.

On the basis of the above relationship of the two genes we may now make the following genic analysis:—

P ₁	♀	Ratanghose	X	♂	Burma
		aa r _o r _o			AA R _o R _o
		Colourless roots.			Coloured roots.
F ₁		Aa R _o r _o Coloured roots.			
F ₂	Genotypes.		Behaviour in F ₃		
1	AA R _o R _o	Coloured roots	Breeds true.		
2	AA R _o r _o	" "	Splits in 3 coloured: 1 colourless		
2	Aa R _o R _o	" "	" "	3	" : 1 "
4	Aa R _o r _o	" "	" "	9	" : 7 "
1	AA r _o r _o	Colourless roots	} All of these give colourless roots.		
2	Aa r _o r _o	" "			
1	aa R _o R _o	" "			
2	aa R _o r _o	" "			
1	aa r _o r _o	" "			

It will be noted that all the F₂ genotypes which produce no colour on roots are expected to breed true in the F₃ generation. This is what actually happens.

The F₂ plants with coloured roots breed in three ways in F₃: (1) breeding true (**AA R_oR_o**), (2) splitting in the ratio of 3 coloured: 1 colourless, (**AA R_or_o** + **Aa R_oR_o**), and (3) segregating in the ratio of 9 coloured: 7 colourless (**Aa R_or_o**). The actual breeding behaviour of the 19 F₂ lines, utilised for F₃ study, conforms to this expectation. Further, the actual distribution of the various genotypes of the 19 F₂ lines approximates very closely to the expected frequencies as will be seen from the Table IV.

TABLE IV
Genotype distribution of the 19 F₂ cultures from
♀ Ratanghose X ♂ Burma

Genotype	AA R ₀ R ₀	AA R ₀ r ₀ Aa R ₀ R ₀	Aa R ₀ r ₀	Total	X ²	P
Observed	2	9	8	19		
Expected	2.11	8.44	8.44	18.99		
Deviation	-0.11	0.56	-0.44	-0.01		
X ² /m	0.00573	0.03715	0.02294		0.066	.989- 0-0.5

Summary

A Burmese variety, No. 355, develops colour on roots when exposed to sunlight. The character is governed by two genes **A** and **R₀**, complementary in action. **A** is the principal gene without which no colour can be produced in any part of the plant; while **R₀** is the specific gene which produces colour on the roots in the presence of **A**.

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* Originals not seen.

A CHEAP DEVICE FOR USING SAFETY RAZOR BLADES FOR MICROTOME SECTIONS

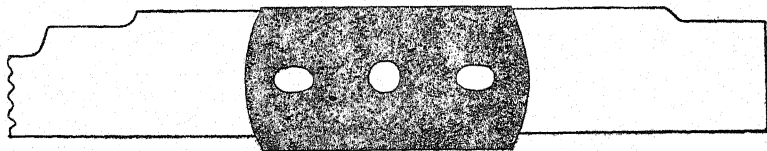
BY

H. R. BHARGAVA.

Received for publication on 1st February 1934.

For getting good microtome sections it is necessary that the edge of the knife should be very sharp, and the sharpening of a microtome knife requires considerable skill and labour. It is the dull knife which causes most of the trouble in cutting. The safety razors now in general use have cheap blades of thin steel with a uniformly sharp edge. The price of a blade is so low that it can be discarded as soon as the edge becomes dull and thus the labour of sharpening is dispensed with. A blade-holder is not absolutely necessary.

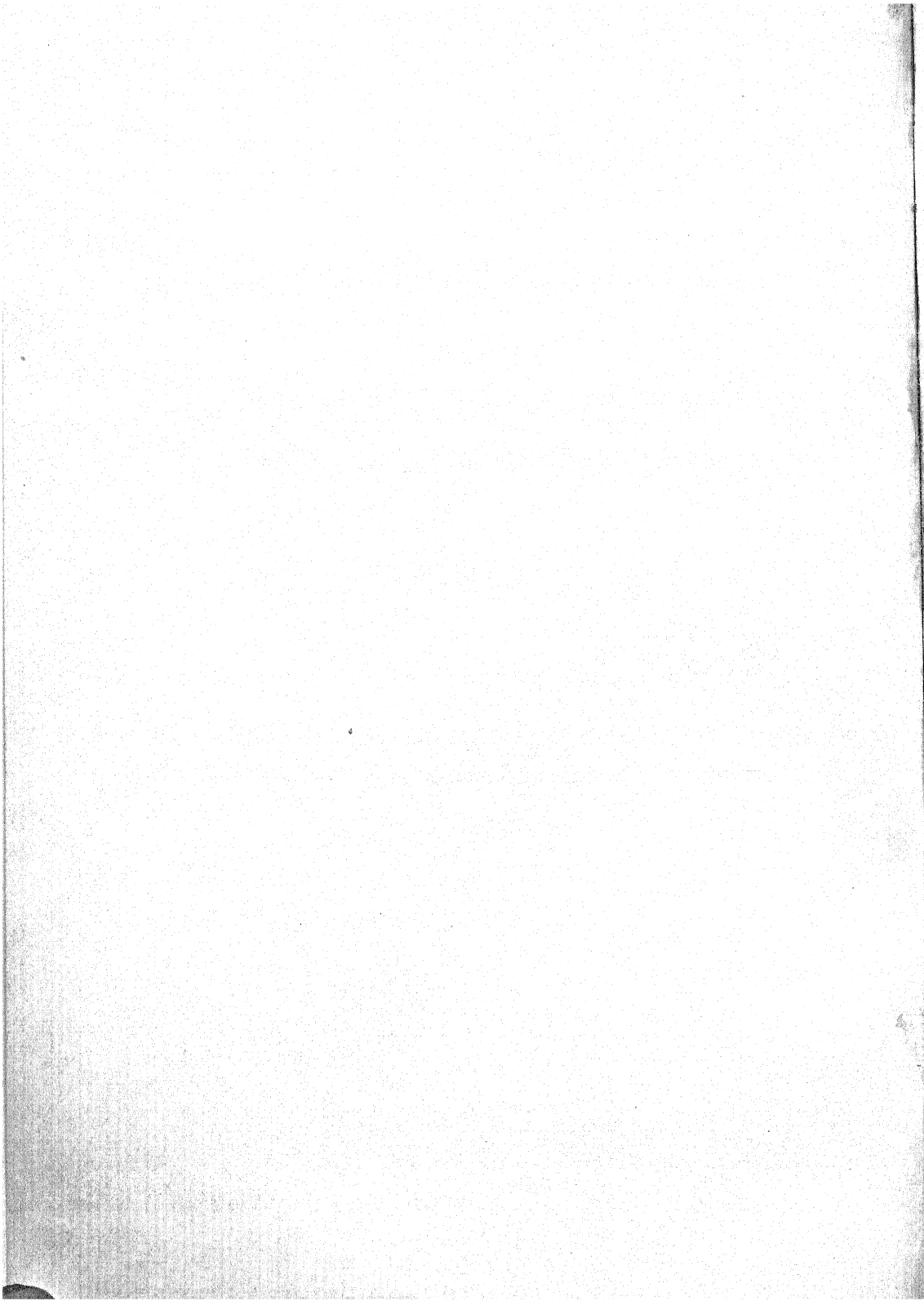
The author suggests melting some hard paraffin (about 60°C. melting point) on an old knife with a plane surface. A clean safety razor blade is then slipped on to it in the middle and the knife is then at once plunged into a dish of cold water. As the paraffin solidifies the blade sticks fast to the knife. The edge of the blade should project only a little beyond the dull edge of the knife and should be parallel with it; if the edge of the blade projects too far there will be less rigidity and it would become very difficult or rather impossible to get uniformly thin sections. Care should also be taken that the amount of paraffin in between the blade and the knife is not too much and is evenly spread. In this laboratory we have tried this method for more than two years and sections of flowers as thin as 4 microns have been cut without any flaw.



In the figure given above is shown a safety razor blade stuck by this method on a knife used with the Cambridge rocking microtome. The knife is not as broad as the blade, therefore some portion of the blade is projecting out on the lower side but this causes no hindrance in cutting the sections.

I am greatly indebted to Dr. P. Maheshwari at whose suggestion this method was tried.

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REVIEW

Upavana-Vinoda (A Sanskrit Treatise on Arbori-Horticulture) by Girija Prasanna Majumdar, M.Sc., B.L., Professor of Botany, Presidency College, Calcutta. (Published by Satis Chandra Seal, M. A., B. L., Honorary General Secretary, The Indian Research Institute, 55, Upper Chitpore Road, Calcutta, 1935, Price Rs. 2-8-0 or 4s. 3d.).

Professor Majumdar has done a service by translating this Sanskrit treatise on Arbori-Horticulture. Most people possess a vague idea what the ancient India had in Positive Science, but they have not the necessary knowledge of Sanskrit nor time to read the original text. Professor Majumdar's book will be welcomed by these people.

As to the book itself, it contains definite empirical instructions about the various processes in arbori-horticulture. Many of these are interesting. One can understand the underlying scientific principles of some of these instructions, although the whole thing is shrouded in language which smacks of superstition. Thus, for example, one can understand the significance of applying cowdung or cooked meat to the ground, but what can be the meaning of the following process described in the section of sowing of seeds (page 14) and who can afford it?

"To ensure inflorescence, etc., Varahamihira directs that the seeds before being sown should be treated as follows:—The seeds should be taken up in the palm greased with ghee and thrown into milk; on the day following the seeds should be taken out of the milk with greased fingers and the mass separated into single seeds. This process is to be repeated on 10 successive days. Then the seeds are to be carefully rubbed with cowdung, and afterwards steamed in a vessel containing the flesh of hogs or deer. Then the seeds are to be sown with the flesh, with the fat of the hogs added in a soil previously prepared by being sown with sesame and dug up or trodden down, and then to be sprinkled daily with water mixed with kshira (milk)."

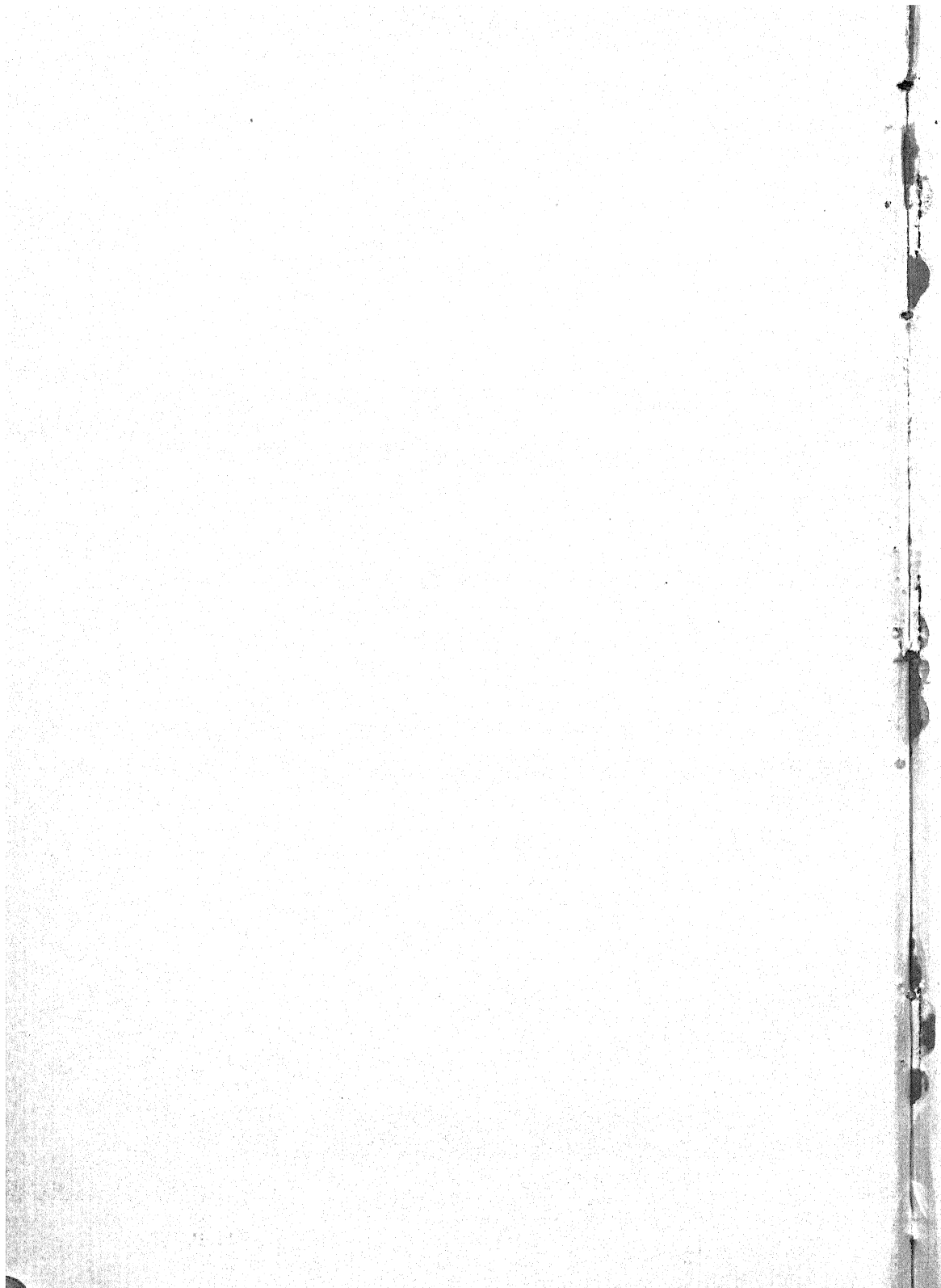
Again, one cannot recommend to the gardeners the following process for the destruction of mice, locusts, ants, etc. :—

"81. If one apprehends danger from mice, locusts, ants, etc., one should utter the following formula (mantra) 108 times, and write it down on the leaf of a tree.

Om svasti kiskindhasthita prakataparakramantarhitarkamandalopajivitasya ca Shrihanumanajnapayati musakapatangapipilikasalahakarakabhanvakakitagandhikanivahairnasthatavyam. Ajnamatikramamanasya sariranigraha samavartayati. Tasya vanarasimhasya kramamanasya sagaram. Kaksantaragato vayur-jumuta iva nardati. Hum phat namah " (page 75).

Whether one believes all that the book says or not, one gets a few hours' pleasure in reading the book. All lovers of Sanskrit lore should welcome the book and should be thankful to the Indian Research Institute for that pleasure.

P. P.



The Journal of the Indian Botanical Society

(Formerly "The Journal of Indian Botany")

VOL. XIV

SEPTEMBER, 1935

No. 3

STUDIES IN ABSORPTION AND TRANSPIRATION—II

Cut Shoots treated with different concentrations of Sodium chloride, Potassium nitrate and Formalin solutions

BY

T. EKAMBARAM

AND

I. MADHUSUDANA RAO

Received for publication on 1st July, 1933.

Introduction

A previous paper on this subject dealt with the effects of 20 per cent. formalin on absorption and transpiration with cut shoots of *Barleria crista* and it was shown that the cortical cells do not take part in the ascent of sap. It was felt, it would be interesting to note the effects of different concentrations of formalin on absorption and transpiration with the cut shoots of the same plant. Moreover, one point in the previous paper needed further elucidation. When 20 per cent. formalin was supplied to the cut end of a shoot of *Barleria*, a fall in transpiration to half the initial rate was noticed before the discoloration in the leaves due to death took place. This fall in the rate was supposed to be caused by the osmotic effect of the formalin solution on the transpiring mesophyll cells of the leaves but was not explained as such, as there was no conclusive evidence for the same at that time.

If, as was believed, the fall in the rate of transpiration was due to the osmotic effect of the 20 per cent. formalin, any osmotically active solution supplied through the cut end of the shoot should give the same results, and the magnitude should vary with the concentration. For obtaining the osmotic effect only without any other complications, the solute should be non-permeable and non-toxic. If a permeable salt is used, the result obtained should be different from that of the non-permeable salt.

With these points in view, the effects of different concentrations of formalin, sodium chloride and potassium nitrate were studied. Sodium chloride is chosen as it is known that it is practically non-permeable.

Review of previous Literature

Literature on this line of investigation is found to be meagre. Dixon (2), Overton (3), Bose (1) and others who were working on the effects of poisons on transpiration or absorption used different poisons but each poison in a fixed but fairly high concentration, as the main object in their experiments was to kill the cells. Formalin is very well known for its highly poisonous effect on living cells and the higher the concentration of the solution, the greater is its toxicity. It is also recognised that the solutions of formalin are osmotically active and the higher the concentration, the greater is the osmotic value of the solution but this was not studied properly as the osmotic effect of the formalin solution is most often masked by its poisonous effect. But the shrinkage of the cells and other phenomena, often met with in 'bad' fixing of material for the study of cell contents, is obviously due to the osmotic effect of the killing solution. Tarkhan (11), working on animal tissues, has shown that the magnitude of shrinkage depends upon the concentration of the killing fluid.

The effect of sodium chloride solution on transpiration was studied by several workers but all of them were working with rooted plants. Eaton (3) and Meyer (6) noted that a supply of salt solution to the roots reduced transpiration and this they attributed to an increase in cell-sap concentration or they found in some cases the root cells plasmolysed. Schimper observed that the transpiration of plants growing on saline soil is much less than that of plants in ordinary soil. But these results do not help us in the present investigation, as here cut shoots are used, and the salt solution is supplied direct to the cut end.

Methods adopted

The same apparatus, as was already described (5), was used to get automatic records of rate of absorption and rate of transpiration with cut shoots. No modification of the apparatus was found to be necessary.

Material

As the earlier work had been carried out with *Barleria cristata*, the same plant was used in this work. Previous experience with this plant had made it possible to eliminate certain sources of error and had brought out the importance of the effects of two factors, namely, age of the shoot and initial water-content on the results obtained.

The shoot was cut, as before, early in the morning and fixed in the apparatus with the cut end in water. Recording was started a little later allowing some time for the shoot to settle down. In the case of absorption, recording was continued on all occasions till next morning, while in transpiration it was stopped in the evening of the same day.

Formalin was supplied in the following concentrations: 50% 20%, 10%, 5% and 1% to the cut end of the shoot to note their effects on absorption and transpiration. Commercial formalin was diluted to the above concentrations (*i.e.*, to prepare 50 per cent. formalin, 50 cc. of distilled water was added to 50 cc. of the commercial formalin making up 100 cc. of the solution). Sodium chloride solution was used in concentrations of 10%, 5%, 2% and 1% (10 gms. of sodium chloride was dissolved in 100 cc. of distilled water to make 10 per cent. solution) and potassium nitrate in 10% and 5% solutions.

Experimental Results

ABSORPTION.

The rate of absorption of water by the cut shoots was followed throughout the day and night on different occasions. The graphs obtained from these data showed very little difference from one another in their general nature.

One such experiment for the rate of absorption of water by a cut shoot is given in detail below.

Experiment No. 1.—25th April 1931. (Expt. No. 1 of the previous paper, (5).)

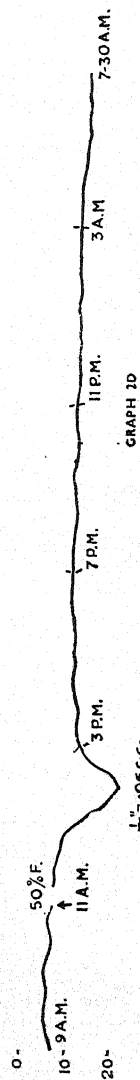
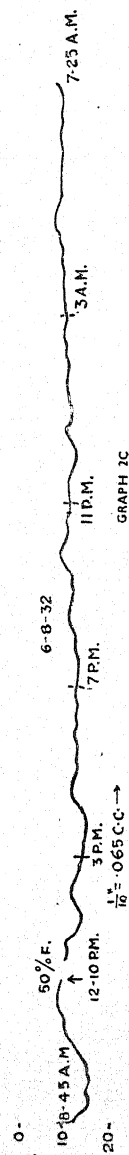
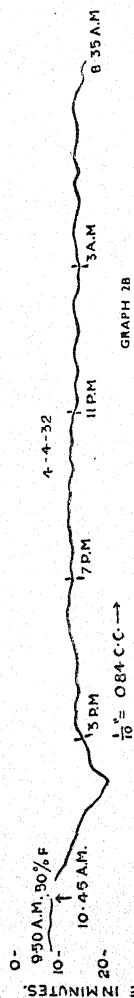
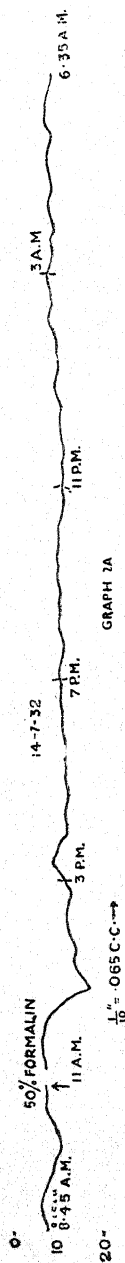
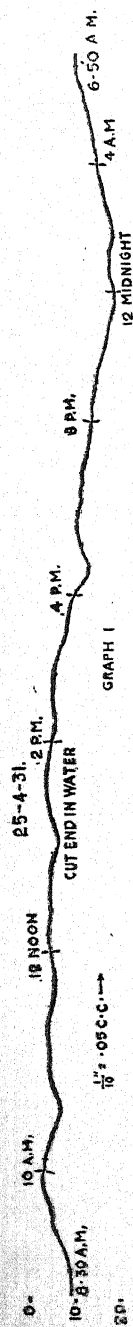
Rate of absorption of water.

Graph 1

Description of the shoot: 1½ ft. long; young; well-growing; 7 pairs of leaves; no branches.

Temperature of the room (T): 87°·6F. at 9 a.m.

Shoot cut at 7-30 a.m. and fixed in the apparatus with the cut end in water. Recording started at 8-30 a.m. Rate quite slow at the beginning but by 10 a.m. it was double the initial rate. Slight variation in the rate till 3 p.m. From 3 p.m. to 5 p.m. rate going down rather rapidly. A slight pause till 6 p.m. and again a continuous decrease in the rate till midnight. Rate going up slowly from



• 1' • 1" • 2' • 3' • 4' • 5' • 6" • 7' • 8' • 9' • 10" • 11' • 12' • 13' • 14'

2 a.m. but rather quickly from 5 a.m. Experiment continued till 6-45 a.m. next morning.

Experiment No. 2-A.—14th July 1932.

50% formalin supplied to the cut end.

Graph 2 A

Shoot: 1 ft. 3 ins. long; old; 4 small branches; about 15 leaves altogether; stem bare up to a height of 10 ins. from the cut end.

Weather: Bright; warm. (*T*): 86° F. at 9-45 a.m.

Shoot cut at 7-15 a.m. and recording from 8-30 a.m. Rate more or less constant, about 5 minutes (M.) for absorbing 0.065 cc. of water. Water removed and 50 per cent. formalin supplied at 10-45 a.m. Rate slowly going down from 11-30 a.m. (6½ M.) to 12-30 p.m. (11 M.) and then a fairly rapid fall. Minimum rate reached by 1-15 p.m. (17 M.). Recovery started at 3 p.m. (15 M.) and complete by 5 p.m. (10½ M.). Recovered rate fairly constant till 9 p.m. and then going down slowly. Again a slight recovery early next morning (8 a.m., 12 M.). Recording continued till 8 a.m. next morning.

11-45 a.m.—Discoloration in some of the leaves. 3 p.m.—All leaves well discoloured. Leaves completely discoloured and curling by next morning.

When 50 per cent formalin was supplied to the cut end, there was no immediate fall. The rate started to go down slowly from about 45 minutes after supplying the poison. It must be remembered from the description of the shoot that the stem was bare up to a height of 10 ins. So the formalin took a fairly long time to reach the leaves and this could be seen by the very gradual fall till then. Once the formalin reached the leaves, the fall was getting more and more rapid. As all the leaves got discoloured, the rate reached its minimum and then the recovery started. This recovered rate was only half the initial rate but it was fairly constant afterwards.

It should also be noted here that the very slow fall in the rate after supplying the cut end with formalin was mostly due to the condition of the shoot which was old. If the shoot was young, tender and well-growing, this fall would have been still greater even from the beginning as is seen in the following experiment.

Experiment No. 2-B.—4th April 1932.

50% formalin supplied to the cut end.

Graph 2 B

Shoot: Young; 1 ft. long; 6 pairs of leaves.

Weather: Bright; warm.

Shoot cut at 7-45 a.m. and recording from 9-50 a.m. Rate quite steady (8 M. for absorbing 0.084 cc.).

50 per cent. formalin supplied at 10-45 a.m. Fall in the rate started immediately and the rate going down rapidly. Rate at 12 noon (14 M.). At 1 p.m. (19½ M.). Minimum rate (21 M.) at 1-45 p.m. Then a rapid recovery in the rate till 3 p.m. (15 M.). Recovery complete by 6 p.m. (13 M.). Recovered rate fairly constant till the end of the experiment.

Slight discoloration in a few leaves at 12-15 p.m. In another hour all the leaves well discoloured.

The shoot was fairly young and so the rate of absorption started going down immediately after the supply of 50 per cent. formalin to the cut end. As the distance between the cut end and the first pair of leaves on the stem was very little, there is no difference in steepness between the earlier and later parts of the curve as seen in Experiment 2-A.

Experiment No. 2-C.—6th August 1932.

50% formalin supplied to the cut end.

Graph 2 C

Shoot: No leaves on the main stem which ended blindly at a height of 3 ins. from the cut end; 2 small slender branches from a height of about 2 ins. from the cut end and 2 fairly long (about 4 ins.) branches from near the top of the main stem; altogether about 15 leaves; old shoot.

Weather: Cloudy till about 12 noon; then bright and warm. (T): 83° F. at 9 a.m.

Shoot cut at 7-45 a.m. and recording from 8-45 a.m. Rate slow to start with (9 a.m., 13 M. for absorbing 0.065 cc.) but slowly increasing. 12-10 p.m., 10 M.

Supplied 50 per cent. formalin at 12-10 p.m. Rate fairly constant till 1 p.m. Then a slow fall. 1-30 p.m., 14 M. A slight recovery bringing the rate to 12 M. at 2 p.m. Again a slow fall with minimum rate of 15 M. at 4 p.m. Final recovery in the rate which kept constant till next morning. This recovered rate was equal to the initial. Rate at 7 a.m. next morning, 11 M. Curve irregular, showing two minima.

By 3 p.m., the poison reached all the leaves. They were discoloured along the veins. Next morning at 8 a.m. the leaves were completely discoloured and were curling slightly. There was no collapse of the branches. Even the petioles had not collapsed. The soil, in which the plant was growing, was quite dry. The plant was not watered properly. Saturation deficit of the shoot must have been higher, but the leaves on the plant did not show signs of wilting when the shoot was cut from it. The shoot itself was old.

*Effect of 50% formalin supplied to the cut end.***Graph 2 D**

The general course of the rate of absorption of a cut shoot with 50 per cent. formalin at the cut end, is represented theoretically in the Graph 2-D, taking the shoot to be mature, and fairly turgid and the leaves arising from a height of 6 ins. from the cut end.

The graph shows that the rate of absorption goes down slowly from the time of supplying the cut end with 50 per cent. formalin. As the poison reaches the leaves, the rate goes down more rapidly. The minimum rate is found to occur with the discoloration of all the leaves. After this, the rate recovers fairly rapidly and the recovered rate is about half the initial rate. The recovered rate keeps constant for sometime.

The curve for the rate of absorption shows three definite phases in its course, from the time of supplying 50 per cent. formalin to the cut end:

- (i) till the formalin reaches the leaves,
- (ii) as the leaf-cells are killed by the formalin, and
- (iii) after the death of the leaf cells.

These points will be considered by a comparison of the 3 graphs 2-A, 2-B and 2-C with the typical one 2-D.

(i) In the first phase, Graph 2-D shows a slow fall in the rate of absorption for sometime, the shoot being mature and fairly turgid with the leaves starting from a height of 6 ins. from the cut end.

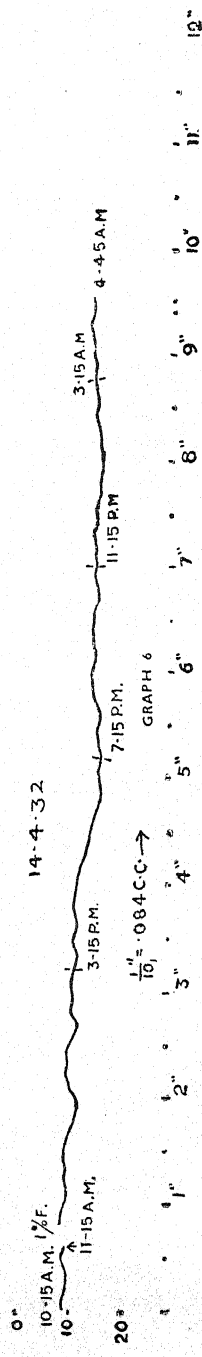
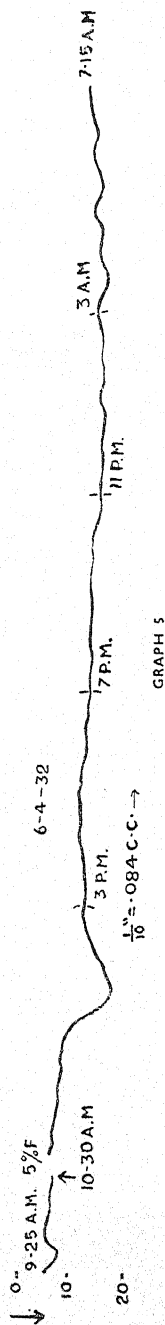
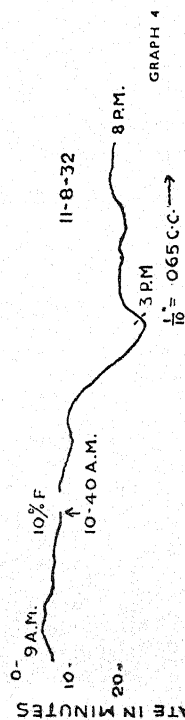
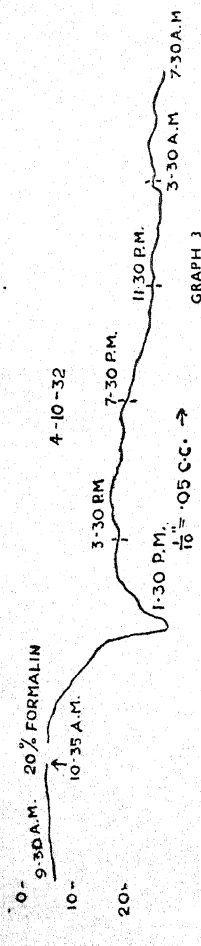
In the graph for Experiment 2-A, this initial fall is not noticeable as the shoot is old.

In the graph for Experiment 2-B, the fall in the rate of absorption starts with the supply of 50 per cent. formalin to the cut end and it goes down fairly rapidly. The shoot is young and turgid.

In the graph for Experiment 2-C, the initial fall is not clear; the shoot is old and the water-content is low.

(ii) The second phase shows a big fall in the rate of absorption in Experiment 2-D, starting sometime after the supply of formalin. The fall itself is big as the shoot is fairly turgid. The minimum rate is obtained with the discoloration of all the leaves.

In Experiment 2-B this fall in the rate starts much earlier. It is difficult to separate the initial fall and later fall. This is because the shoot is young and the leaves start from near the cut end of the shoot. The magnitude of the fall is much greater in this case than in the other three experiments.



In Experiment 2-C, this fall is much less in magnitude than in any of the previous cases. The shoot is quite old and its water-content is low.

In all these experiments, the fall starts with the beginning of discoloration in the leaves and the rate reaches the minimum with the completion of the discoloration of all the leaves.

(iii) The third phase of the curve shows the recovery in the rate of absorption in all cases after the discoloration of the leaves. The recovery is fairly rapid in 2-B and 2-D, where the water-content of the shoot is high and the shoots are not old. In the other two, 2-A and 2-C, the recovery is slow, as the shoots are old and are not quite turgid.

The recovered rate is more than half the initial rate in 2-A and 2-D, where the shoots are not young. In 2-B, the recovered rate is about half the initial rate as the shoot here is young and turgid. In 2-C, the recovered rate is almost equal to the initial rate. Here the shoot is old and the water-content is low.

The recovered rate in all these experiments is fairly constant for a long time.

Experiment No. 3.—4th October 1931. (Experiment No. 2 of the previous paper, (5).)

20% formalin supplied to the cut end.

Graph 3

Shoot: 10 ins. long; young; 6 pairs of large fully turgid leaves.

Weather: Cool; cloudy. (*T*): 82°·8F. at 11 a.m.

Shoot cut at 8-30 a.m., and recording from 9 a.m. (5 M. for absorbing 0·05 cc.).

Supplied 20 per cent. formalin at 10-35 a.m. (4½ M.). Poison observed in the top leaves by the slight discoloration along the veins by 11-30 a.m. (13 M.). Complete discoloration along the veins by 12-30 p.m. (minimum rate of 27 M.). Recovery at 1-15 p.m. (22 M.). At 5 p.m., 16 M. Then a slow fall in the rate. At midnight, 23 M. Experiment continued till 7-30 a.m. next morning. All leaves completely discoloured and curling.

When 20 per cent. formalin was supplied to the cut end, there was a slow fall from about 15 minutes after supplying the poison. The fall soon became rapid and with the discoloration of the leaves, the curve went down fairly steep. This steep fall in the rate was due to the high water-content of the leaves. The recovery was also quick though the weather was cloudy. The recovered rate was not equal to the initial rate. This recovered rate was going down slowly.

Experiment No. 4.—11th August 1932.

10% formalin supplied to the cut end.

Graph 4

Shoot: 10 ins. long; mature; stem bare up to a height of 6 ins.; about 15 small leaves.

Weather: Bright; warm. (*T*): $84^{\circ} \cdot 5^{\circ}\text{F}$. at 10-45 a.m.

Shoot cut at 7-30 a.m. and recording from 9 a.m. Rate more or less constant. At 10 a.m., 7 M. for absorbing 0.065 cc.

Supplied 10 per cent. formalin at 10-40 a.m. (8 M.). No immediate fall in the rate. From 12 noon (9 M.) rate started going down. Rapid fall from 1 p.m. (17 M.). Minimum rate (24 M.) at 2-30 p.m. Then a slow recovery. Recovery complete by 8 p.m. but this rate ($17\frac{1}{2}$ M.) was quite low when compared to the initial rate (8 M.).

Minimum rate obtained with the discoloration of all the leaves. Discoloration started at 12-30 p.m.

Immediately after adding the poison, the rate was not going down visibly. Till 12 noon, the rate was fairly constant though there could be seen a slight sloping of the curve. From 12-15 p.m., the rate was going down quickly. That the poison had reached the leaves at this time could be seen by the discoloration. The fall in the rate of absorption immediately after supplying the cut end was not clear as the formalin solution supplied was of a lower concentration. The regular fall in the rate was obtained with the killing of the leaf-cells. As the killing itself was not rapid due to the low concentration of the formalin, the fall and recovery in the rate of absorption were not rapid.

Experiment No. 5.—6th April 1932.

5% formalin supplied to the cut end.

Graph 5

Shoot: 1 ft. 3 ins. long; not quite mature; 8 pairs of leaves.

Weather: Bright; warm.

Shoot cut at 7-15 a.m. and recording from 9-25 a.m. Rate a little unsteady in the beginning but soon became constant. 6 M. for absorbing 0.084 cc. at 10 a.m., and 7 M. at 10-30 a.m.

Supplied 5 per cent. formalin at 10-30 a.m. Rate going down very slowly till 12-15 p.m. ($8\frac{1}{2}$ M.); then a more rapid fall. Discoloration along the veins in all the leaves by 12-30 p.m. Minimum rate (17 M.) at 1-15 p.m. Then a fairly rapid recovery which was complete by 3 p.m. (12 M.); all the leaves completely discoloured. Recovered rate constant till next morning.

After supplying 5 per cent. formalin to the cut end, there was practically no fall in the rate of absorption for about $1\frac{1}{2}$ hours. As the concentration of the formalin was low, this absence of fall in the rate in the beginning was no surprise. But when the poison entered the leaves, the fall was rapid; the recovery too was fairly rapid. The rapid fall in the rate and equally rapid recovery were here due to the condition of the shoot which was not quite mature and the weather which was bright and warm.

Experiment No. 6.—14th April 1932.

1% formalin supplied to the cut end.

Graph 6

Shoot: 1 ft. 3 ins. long; fairly mature; 7 pairs of leaves.

Weather: Bright; warm. (*T*): 86°F. at 10 a.m.

Shoot cut at 8 a.m. and recording from 10-15 a.m. Rate almost constant (9 M. for absorbing 0.084 cc.).

Supplied 1 per cent. formalin at 11-15 a.m. Rate rather unsteady till about 3 p.m. Then a very slow fall. At 3-15 p.m., the rate was 10 M. Rate going down a little more rapidly. Slight discoloration in two of the lower leaves at 3-15 p.m. Minimum rate at about 6-30 p.m. (15 M.). Discoloration was clear along the veins in the lower leaves by 6-45 p.m. Very slow recovery which continued the whole night. At 4-50 a.m., next morning, the rate was $12\frac{1}{2}$ M.

When 1 per cent. formalin was supplied to the cut end of a shoot, the initial fall in the rate of absorption, before the poison reached the leaves, was not seen. When it reached the leaves, the killing of the leaf cells was not so rapid as in the case of the previous concentrations. The killing was spread over a longer time. Even the recovery was quite slow. It was not certain whether all the cells were killed even by next morning.

Changes in the rate of absorption with a supply of different concentrations of formalin to the cut end.

In all concentrations of formalin, a fall in the rate of absorption is seen during the killing of the leaf cells and a recovery in the rate after the death of the cells. The recovered rate is fairly constant for a long time.

The magnitude of the initial fall in the rate of absorption, as the formalin goes up the stem, depends on the concentration of formalin, age and the initial water-content of the shoot. In higher concentrations such as 50 per cent. formalin even with mature turgid shoots, this fall is quite clear. In young and turgid shoots, even

with lower concentrations such as 20 per cent. and 10 per cent. formalin, this fall is noticeable.

The nature of the curve for the later big fall depends on the concentration of the formalin, the age and the water-content of the shoot. With similar young and turgid shoots, the fall is bigger and more rapid initially in higher concentrations. With young and highly turgid shoots, the fall is quite big even in lower concentrations but it is more rapid in higher concentrations.

The recovery in the rate of absorption is more rapid in higher concentrations and recovered rate is about half the initial rate in all concentrations using similar mature turgid shoots. With young and turgid shoots in higher concentrations of formalin, the recovered rate is about half the initial rate but with the same kind of shoots in the lower concentrations, the recovered rate is less than half the initial rate.

The effects of various concentrations of a purely osmotic solution are shown by the following experiments.

Experiment No. 7-A.—7th October 1931.

Supplied 10% sodium chloride solution to the cut end.

Graph 7 A

Shoot: 8 ins. long; young; a few small branches; about 20 leaves.

Weather: Warm; dull.

Shoot cut at 8 a.m. and recording from 10-45 a.m. Rate rapid and constant (4 M. for absorbing 0.05 cc.). Supplied 10 per cent. sodium chloride solution at 12 noon. Fall in the rate started immediately and was rapid. Rate at 1 p.m., 13 M. Leaves fairly drooping by this time. Rate a little steady between 1 p.m. and 2 p.m., but again going down rapidly till late in the night. Rate at 12 midnight, 60 M. Then a slight increase in the rate. At 7 a.m. next morning, 50 M.

Leaves completely drooping by next morning. Main shoot and small branches shrunken at their upper nodes; some of the smaller branches hanging down. Shoot tip also hanging down due to collapse at the top nodes.

When an osmotic solution of a non-permeable salt of a fairly high concentration, as 10 per cent. sodium chloride solution was supplied to the cut end of a shoot, the rate of absorption goes down continuously. But the magnitude and rate of fall depend on the nature of the shoot, *i.e.*, the age of the shoot and its water-content. The younger the shoot and the higher the water-content, the greater will be the fall in the rate of absorption.

The following experiment shows to what extent the fall in the rate of absorption after supplying the cut end with 10 per cent. sodium chloride solution depends on the age of the shoot and its water-content.

Experiment No. 7-B.—27th October 1931.

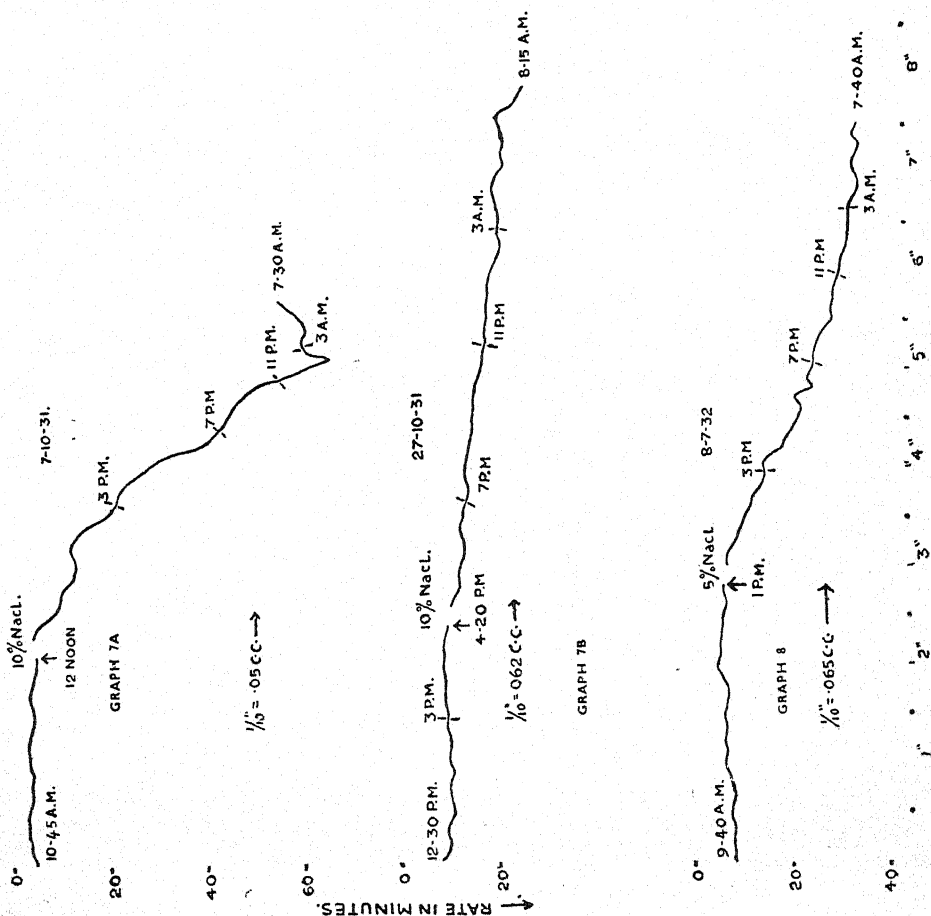
Supplied 10% sodium chloride solution to the cut end.

Graph 7 B

Shoot: Mature; $1\frac{1}{4}$ ft. long; 8 pairs of leaves; a small branch at the first node.

Weather: Cloudy.

Shoot cut at 8 a.m. and recording from 12-30 p.m. Rate fairly constant (10 M. for absorbing 0.062 cc. of water).



Supplied 10 per cent. sodium chloride solution at 4-20 p.m. Fall in the rate started immediately and the rate was going down continuously at a much less rapid rate than in the previous experiment. Rate at 8-15 a.m., next morning, 26 M. All the leaves drooping. Some of the lower leaves slightly discoloured at the tips and margins.

In this case, the shoot was mature and the water-content appeared to be low. Thus the fall in the rate of absorption was not so rapid nor of the same magnitude as in the previous experiment.

Experiment No. 8.—8th July 1932.

Supplied 5% sodium chloride solution to the cut end.

Graph 8

Shoot: 1 ft. 3 ins. long; well-growing; about 20 small leaves; a few small branches; young.

Weather: Cool; dull. (*T*): 83°·5F. at 1 p.m.

Shoot cut at 7-30 a.m. and recording from 9-40 a.m. Rate slowly going up. At 11 a.m., 7 M. for absorbing 0·065 cc. At 1 p.m., 6½ M.

Supplied 5 per cent. sodium chloride solution at 1 p.m. Fall in the rate started immediately even with 5 per cent. solution and the rate going down rapidly till 9 p.m. (20 M.). Then a more gradual fall and the curve quite flat from 3 a.m. next morning. Experiment stopped at 7-40 a.m. next morning.

By 5 p.m., all leaves completely drooping. Even the small branches hanging down by next morning. Some of the lower leaves discoloured at the margins and tips.

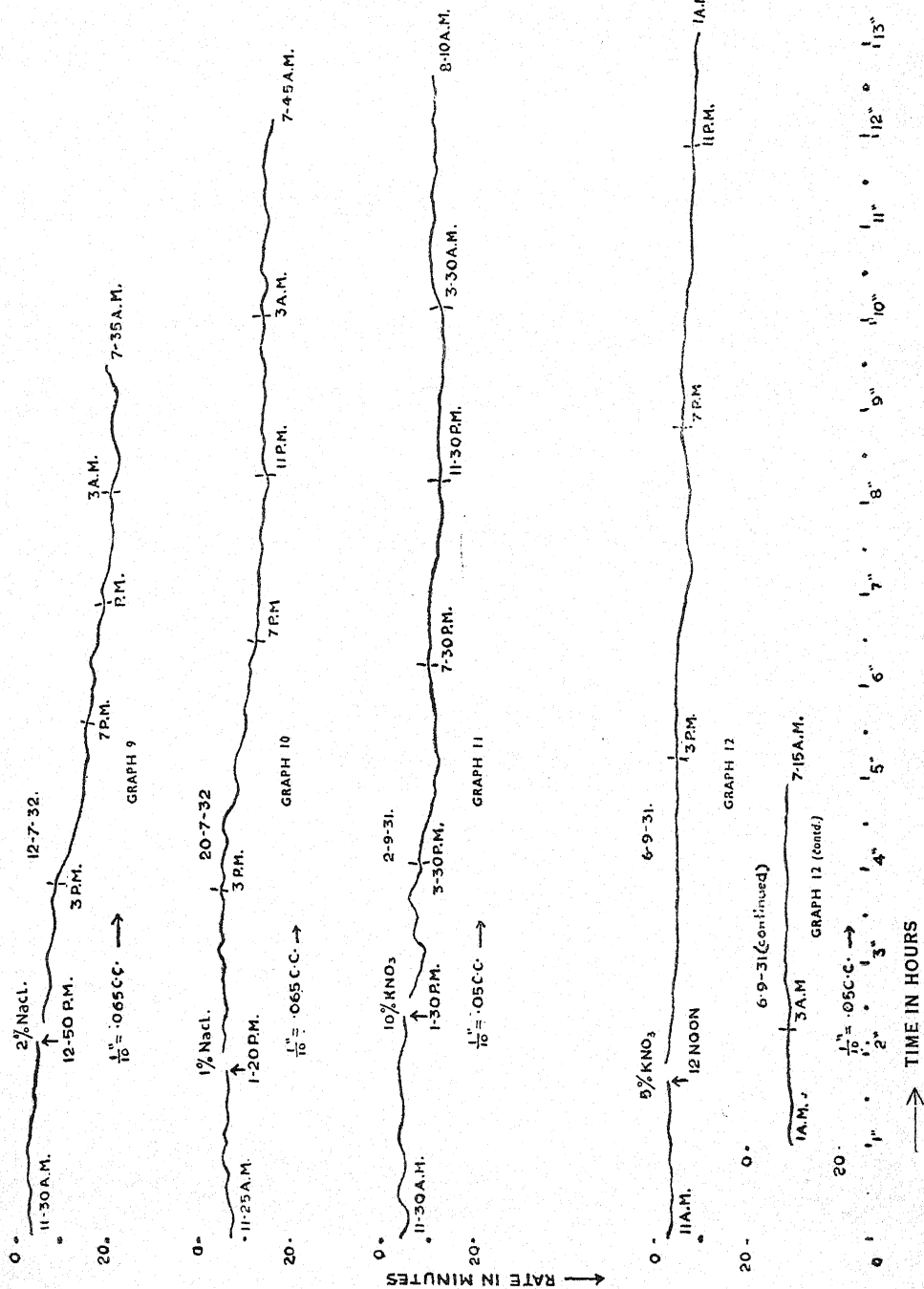
Here the fall in the rate of absorption is not to the same extent as in Experiment 7-A though the shoots were fairly similar. This experiment shows that the fall depends also upon the concentration of the osmotic solution supplied to the cut end.

Experiment No. 9.—12th July 1932.

Supplied 2% sodium chloride solution to the cut end.

Graph 9

Shoot: 10 ins. long; fairly mature; a few small branches; about 24 small leaves.



Shoot cut at 7-15 a.m. and recording from 11-30 a.m. Rate constant (about 4 M. for absorbing 0.065 cc.). At 12-50 p.m., 5 M.

Supplied 2 per cent. sodium chloride solution at 12-50 p.m. Rate going down very slowly till 3 p.m. (9 M.). More rapid fall till 11 p.m. Then fairly steady till 4 a.m. next morning. Experiment stopped at 7-35 a.m. next morning (20 M.).

No drooping of the leaves; leaves turgid even by next morning.

With 2 per cent. sodium chloride solution at the cut end, the fall in the rate of absorption is much reduced. After the salt solution enters the leaves, its effect on the rate of absorption could be seen. Thus the rate goes down fairly quickly and continuously from 3 p.m. in this experiment. It must be noted that, when the cut end is kept in water throughout the experiment, the rate goes down slowly from about 3-30 p.m. (Experiment No. 1). So the fall in absorption in this experiment from 3 p.m. was only partly due to the effect of the salt solution. The rate of absorption had not gone down to the same extent or with the same rapidity with 2 per cent. sodium chloride solution as it was with 5 per cent. solution.

Experiment No. 10.—20th July 1932.

Supplied 1% sodium chloride solution to the cut end.

Graph 10

Shoot: 10 ins. long; fairly mature; about 15 leaves; leaves starting from a height of 6 ins. from the cut end.

Weather: Bright; warm. T: 11-30 a.m., 89°F.

Shoot cut at 7-15 a.m., and recording from 11-25 a.m. Rate quite constant from 12 noon (6 M. for absorbing 0.065 cc.).

Supplied 1 per cent. sodium chloride solution at 1-20 p.m. No fall in the rate till 3-30 p.m. But a slight increase at 3 p.m. (4½ M.). A slow fall from about 4 p.m., till 7 p.m. Then the rate was steady till next morning. At 7 a.m., the rate was 14 M. Experiment stopped at 7-50 a.m.

With 1 per cent. sodium chloride solution at the cut end, the rate was following its normal course till 3-30 p.m., when the rate started going down slowly till next morning. In the case of a shoot with its cut end in water throughout the experiment, the rate of

absorption goes down slowly from about 3 p.m., but next mornnig, it recovers. This recovery in the rate was not seen with 1 per cent. sodium chloride solution at the cut end of the shoot.

Changes in the rate of absorption with a supply of different concentrations of sodium chloride solution to the cut end.

When a solution of sodium chloride is supplied to the cut end, as the solution goes up the stem, a fall in the rate of absorption starts, the magnitude of the fall depending on the concentration of the solution supplied and the condition of the shoot. The fall gets more rapid when the solution enters the leaves and when all the leaves are drooping, the fall is less rapid and the rate may tend to get steady.

With 10 per cent. sodium chloride solution, the initial fall is fairly rapid depending on the condition of the shoot. In a young turgid shoot, this fall is quite rapid. The magnitude of this fall decreases with lower concentrations and in 1 per cent. solution, it is absent.

The later fall in the rate of absorption, as the solution enters the leaves, is not so marked as in the formalin solutions. The same initial fall continuous but is slightly more rapid. The curve for fall in absorption is continuous and cannot be separated into an initial and a later portion as was done with formalin curves.

The fall in the rate of absorption, after the solution had entered all the leaves, is not so rapid and the rate tends to get steady. With 10 per cent. sodium chloride solution, this tendency for the rate to get steady is not quite clear though the rate of fall is reduced when the shoot is old.

With 5 per cent. solution, the rate gets steady after all the leaves have shown signs of drooping. With still lower concentrations, the rate gets steady after sometime.

The effects of two different concentrations of potassium nitrate, which is permeable, on the rate of absorption were also studied. In the previous experiments, the salt used (sodium chloride) is non-permeable. So the effect on absorption with the sodium chloride solution was all due only to the osmotic effect of the solution on the cells. But with potassium nitrate solution, there is a further complication due to its permeable nature. How this factor affects the results will be considered in the following experiments.

Experiment No. 11.—2nd September 1931.

Supplied 10% potassium nitrate solution to the cut end.

Graph 11

Shoot: 1 ft. long; 7 pairs of leaves; green; no branches.

Weather: Warm; bright.

Shoot cut at 8 a.m. and recording from 9-20 a.m. Rate slow at the beginning; but soon rapid. Rate at 10 a.m., 3 M. for absorbing 0.05 cc. Very slow fall in the rate from 10 a.m. Rate at 1 p.m., 5 M.

Supplied 10 per cent. potassium solution at 1-30 p.m. An immediate slow fall till 2 p.m. (9 M.). Then a slight rise in the rate continuing till 3 p.m. (6 M.). Again a very slow fall till 5-30 p.m. (13 M.). Then the rate was fairly constant varying within small limits. Rate at 8 a.m. next morning, 11 M.

Visible drooping of the leaves by 4-30 p.m., but much less than in the experiments with 10 per cent. sodium chloride solution. Leaves discoloured in patches. By next morning, lower leaves completely discoloured excepting at the veins. Upper leaves discoloured in patches.

With a supply of 10 per cent. potassium nitrate solution to the cut end, the rate of absorption was going down continuously but very slowly. The rate was more or less steady for sometime but afterwards there was a slow fall. Again there was recovery in the rate to a slight extent next morning.

Experiment No. 12.—6th September 1931.

Supplied 5% potassium nitrate solution to the cut end.

Graph 12

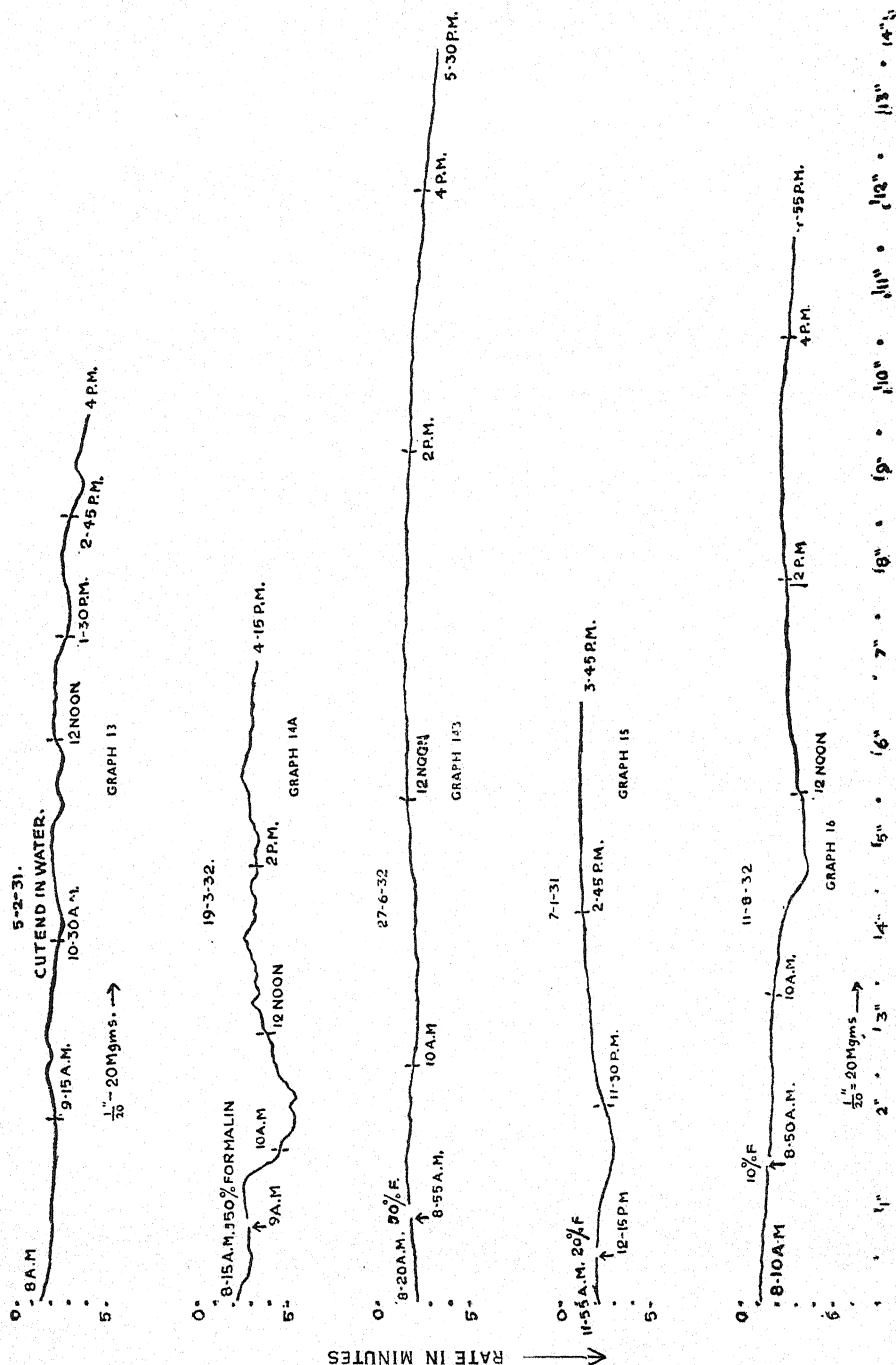
Shoot: 1 ft. long; well-growing; 7 pairs of large green leaves.

Weather: Warm; bright. T: 88°F. at 3 p.m.

Shoot cut at 8 a.m. and recording from 10 a.m. Rate, rapid and constant (3½ M. for absorbing 0.05 cc.).

Supplied 5 per cent. potassium nitrate solution at 12 noon. A very gradual fall in the rate. Rate at 4 p.m., 6 M. Then a quicker fall till 6 p.m. A slight recovery for a short time at 6 p.m. and then again a gradual fall. Rate at 7-15 p.m., next morning, 10 M.

Slight drooping of the leaves at 3-30 p.m. Discoloration not yet seen in any of the leaves. Small patches of discoloration in some of the lower leaves by next morning.



With 5 per cent. potassium nitrate solution at the cut end, the rate of absorption was going down very slowly with small occasional oscillations. There was a tendency for the rate to get steady.

The effect on absorption of supplying potassium nitrate solution to the cut end of a shoot is similar to that of sodium chloride solution to some extent. The fall in the rate of absorption is much less in magnitude and much slower. Also the curve instead of going down continuously gets flat sometimes or even rises up for a short time. The difference in the effects of sodium chloride solution and potassium nitrate solution on transpiration is more evident as will be seen in the experiments on transpiration.

Transpiration

By comparing the effects on transpiration of supplying formalin solution, with those of sodium chloride and potassium nitrate solutions, we will be able to understand more clearly how formalin affects transpiration when it reaches the leaves.

The methods followed were the same as described in the previous paper (5). The shoot was cut in the morning and fixed in the apparatus with the cut end in water. Recording was started an hour later so that in the meantime the shoot might get over the effects of cutting and fixing in the apparatus. Rate of transpiration with the cut end in water was studied on different days under fairly similar conditions and the records were found to be similar in their nature. One such experiment is given in detail.

Experiment No. 13.—5th February 1931. (Experiment No. 7 of paper (5).)

Rate of transpiration with the cut end in water.

Graph 13

(Rate of transpiration means time taken for transpiring 20 mgms. of water).

Shoot: $1\frac{1}{2}$ ft. long; 6 pairs of leaves; not mature.

Weather: Warm; bright. T: 80°F. at 2-30 p.m.

Shoot cut at 7-15 a.m. and recording from 8 a.m. Rate almost constant except for slight variations till 3 p.m. Then a slow fall. At 4 p.m., the rate was one-third the rate at 8 a.m.

The effect on transpiration of supplying different concentrations of formalin to the cut end will be considered now.

Experiment No. 14-A.—19th March 1932.

50% formalin supplied to the cut end.

Graph 14 A

Shoot: 1 ft. long; young; 6 pairs of small leaves; long internodes: first pair of leaves at a height of 3 ins. from the cut end.

Weather: Bright; warm.

Shoot cut at 7-30 a.m. and recording from 8-10 a.m. Rate at the the beginning was $2\frac{1}{2}$ M. Rate constant from 8-40 a.m. (3 M.).

Supplied 50 per cent. formalin at 9 a.m. Rate slightly on the increase till 9-30 a.m. ($2\frac{1}{2}$ M.). Then a rapid fall till 10 a.m. ($4\frac{1}{2}$ M.). No discoloration in any of the leaves. Rate still going down but rather slowly. Rate at 10-30 a.m., ($5\frac{1}{2}$ M.). Discoloration along some of the veins in the lower leaves. A decided increase in the rate from 11 a.m. Discoloration noticed in the upper leaves also. Rate at 12 noon (4 M.). It was still increasing. At 1 p.m., (3 M.). Then steady for sometime. Leaves well discoloured along the veins and discoloration spreading. A very gradual fall in the rate. Experiment stopped at 4-15 p.m. ($3\frac{1}{2}$ M.).

When 50 per cent. formalin was supplied to the cut end, the rate of transpiration was not affected till the formalin reached the leaves. As the formalin entered the leaves, there was a fall in the rate of transpiration. With the first discoloration of the leaves, the fall in the rate slowed down and there was a decided increase in the rate with a visible discoloration in all the leaves. The recovered rate keeps constant for some hours.

That the fall in the rate of transpiration with 50 per cent. formalin at the cut end depends also on the age of the shoot and its water-content, is shown by the following experiment.

Experiment No. 14-B.—27th June 1932.

50% formalin supplied to the cut end.

Graph 14 B

Shoot: Mature; 1 ft. long; leaves from a height of 6 ins. from the cut end; a few small branches; altogether about 15 mature leaves.

Weather: Bright; warm. T: 86°F. at 8-20 a.m.

Shoot cut at 7-45 a.m. and recording from 8-20 a.m. Rate quite constant (2 M.).

Supplied 50 per cent. formalin at 8-55 a.m. Rate at 9-30 a.m. ($1\frac{1}{2}$ M.). A very gradual fall in the rate from 9-30 a.m., till 10-30 a.m. ($2\frac{1}{2}$ M.). Then a slow recovery in the rate till 1 p.m. ($1\frac{1}{2}$ M.). Again a very slow fall in the rate. At 5-30 p.m. (3 M.).

First discoloration seen in some of the leaves along the midrib at 10-15 a.m., rate at its minimum. Leaves well discoloured along the veins by 11-15 a.m., rate also recovered. Discoloration spreading to other parts of the lamina; transpiration high. Leaves more or less completely discoloured; rate was constant. Leaves completely discoloured and curling by 5 p.m., smaller branches hanging down; a very slow fall in the rate.

Experiment No. 15.—7th January 1931. (Experiment No. 8 of paper (5).)

20% formalin supplied to the cut end.

Graph 15

Shoot: Young; about 10 ins. long; 6 pairs of leaves.

Weather: Bright.

Shoot cut at 8-30 a.m. and recording started at 11-55 a.m. Rate constant (2 M.).

Supplied 20 per cent. formalin at 12-15 p.m. A slow fall in the rate from 12-30 p.m. Minimum rate at 1-5 p.m. (3 M.). Rate becoming rapid again. Equal to the initial rate at 1-30 p.m. Continued to quicken though slowly till the end of the experiment.

Discoloration noticed in the leaves at about 1-15 p.m. Leaves completely discoloured by 3-30 p.m.

With 20 per cent. formalin at the cut end, the fall in transpiration is not as high as with 50 per cent. formalin partly because of the lower concentration used and partly of the condition of the shoot.

Experiment No. 16.—11th August 1932.

10% formalin supplied to the cut end.

Graph 16

Shoot: Fairly mature; 8 ins. long; stem bare up to a height of 6 ins. from the cut end; about 15 small leaves.

Weather: Bright; warm. T: 84°F. at 10-45 a.m.

Shoot cut at 7-15 a.m. and recording from 8-10 a.m. (1¼ M.). Rate more or less constant. (1½ M.) at 8-50 a.m.

Supplied 10 per cent. formalin at 8-50 a.m. Rate steady till 10 a.m. (1¼ M.). Rate going down from 10-30 a.m. Minimum rate at 11 a.m. (3¼ M.). Recovery started at 11-15 a.m., discoloration seen in the lower leaves along the veins. Recovery slow but complete by 3-5 p.m. (2 M.). Recovered rate not equal to the initial rate. Rate again going down slowly from 3 p.m. Leaves well discoloured by 4-55 p.m. (3 M.).

As the stem was bare up to a height of 5 ins. from the cut end, the fall in the rate of transpiration started only 1½ hours after supplying the cut end with the formalin. The recovery in this experiment was very slow. As the shoot was fairly mature, the magnitude of the fall or the recovery was not high.

Experiment No. 17.—8th April 1932.

5% formalin supplied to the cut end.

Graph 17

Shoot: 10 ins. long; young; 6 pairs of leaves.

Weather: Warm; bright. T: 89°F. at 11 a.m.

Shoot cut at 8 a.m. and recording from 8-20 a.m. Rate constant. At 9 a.m. (2½ M.).

Supplied 5 per cent formalin at 9 a.m. Rate steady till 10 a.m. Then a slow fall in the rate. More rapid from 10-30 a.m., slight discoloration along the veins in the lower leaves. Minimum rate at 11 a.m. (4½ M.); discoloration seen in all the leaves. Recovery started immediately and was fairly rapid. Leaves well discoloured along the veins by 12 noon (2½ M.). A slow recovery still continued. Constant rate (2 M.) from 1 p.m., till the end of the experiment, *i.e.*, 4-50 p.m., leaves completely discoloured except at the margins and the lower leaves curling.

The fall in the rate of transpiration with 5 per cent formalin was delayed for about an hour and a half. The fall in the rate in this case was much more than in the previous experiment with 10 per cent. formalin to the cut end, as the shoot was quite young here. In young shoots with a fairly high water-content, the recovery will be rapid, of course depending on the weather conditions and the recovered rate in the beginning may be higher than the initial rate.

Experiment No. 18.—15th April 1932.

1% formalin supplied to the cut end.

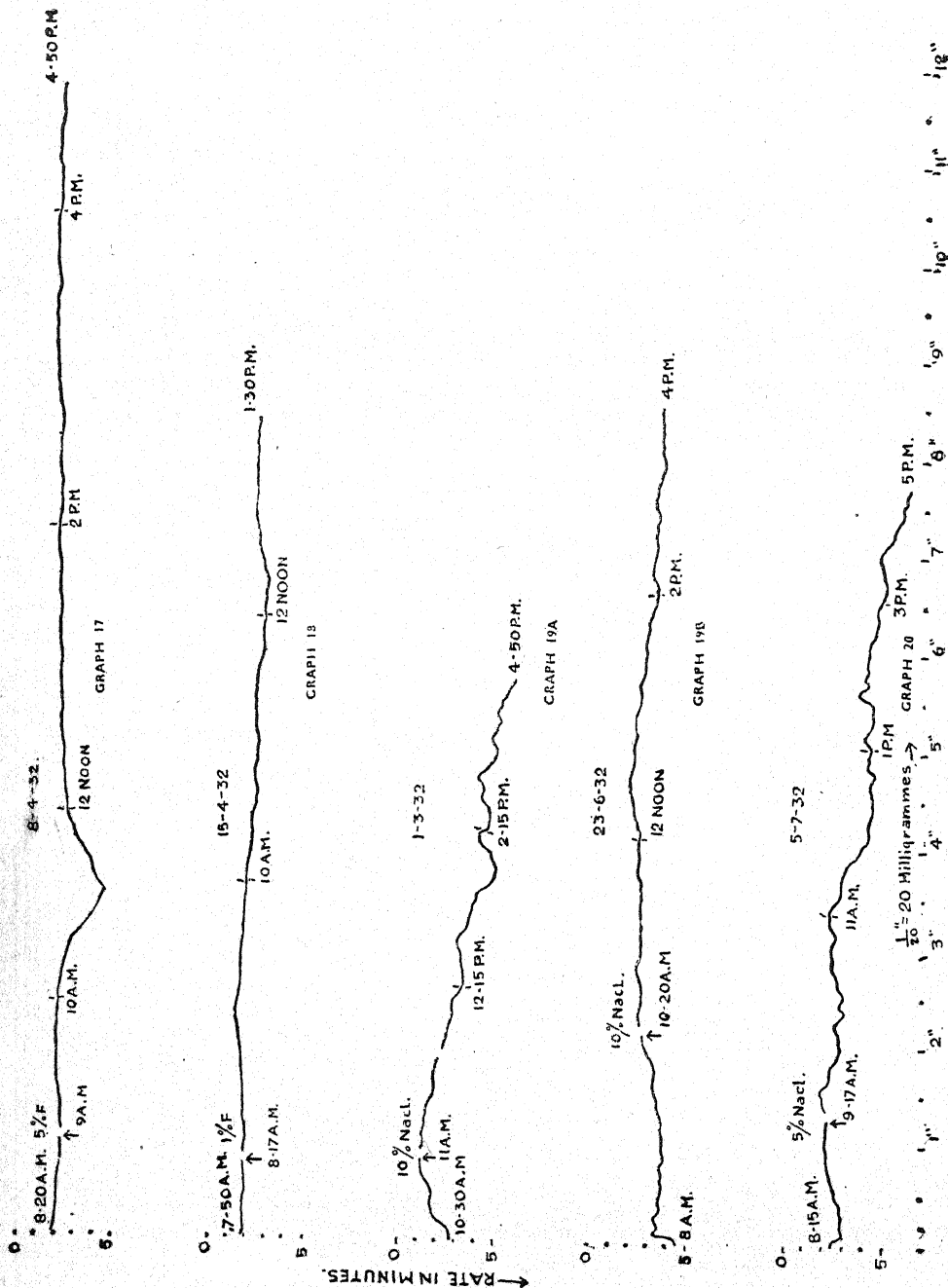
Graph 18

Shooting: 10 ins. long; mature; about 6 pairs of leaves.

Weather: Warm; bright. T: 90°F. at 11 a.m.

Shoot cut at 7-15 a.m. and recording started at 7-50 a.m. Rate constant (2 M.).

Supplied 1 per cent. formalin at 8-17 a.m. Rate following its normal course. A higher rate between 9 a.m. and 9-30 a.m. (1½ M.). Rate at 12 noon (2½ M.). Again a slight increase in the rate at 1 p.m. (2 M.). Experiment stopped at 1-30 p.m. Lower leaves well discoloured along the veins by 12 noon. By 1-30 p.m., all the leaves well discoloured along the veins. Lower leaves more fully discoloured.



With 1 per cent. formalin at the cut end, there was no fall in the rate of transpiration. The rate was keeping fairly constant throughout. The killing was very gradual and so the increase in the rate with the killing was not clear as it was spread over a long time.

Effects on transpiration, when different concentrations of formalin are supplied to the cut ends of shoots.

All the concentrations of formalin, except 1 per cent. solution, used in the above experiments cause a fall in the rate of transpiration as the solution reaches the leaves. The magnitude of the fall depends on the concentration of the formalin solution used. The higher the concentration, the greater is the fall. A recovery in the rate of transpiration is brought about with the death of the leaf-cells. This recovered rate is almost always equal to the initial rate, or even higher.

The effects of different concentrations of sodium chloride solution on transpiration will be considered in the following experiments.

• **Experiment No. 19-A.**—1st March 1932.

10% sodium chloride solution supplied to the cut end.

Graph 19 A

Shoot: 10 ins. long; young; 6 pairs of leaves.

Weather: Cool; bright. T: 81°F. at 11 a.m.

Shoot cut at 8 a.m., and recording from 10-30 a.m. Rate slow to start with but rapidly increasing. At 11 a.m. ($1\frac{1}{4}$ M.).

Supplied 10 per cent. sodium chloride solution at 11 a.m. Rate slowly going down from about 11-20 a.m. Lowest pair of leaves drooping by 11-25 a.m. Rate continuously going down with slight pauses for a short time. At 12-15 p.m. ($4\frac{1}{4}$ M.). By 3-30 p.m., lower leaves completely hanging down. Discoloration of the first pair of leaves at the tips and margins. Top-most leaves flaccid but not completely drooping. Rate at 4-50 p.m. (6 M.).

With 10 per cent. sodium chloride solution at the cut end, a continuous fall, starting from about 15 minutes after supplying the salt solution, was obtained. That the fall started only after the solution reached the leaves could be clearly seen from the result. Much more clearly could it be observed in the following experiment where a mature shoot with the stem bare of leaves up to a height of 5 ins. from the cut end was used.

Experiment No. 19-B.—23rd June 1932.

10% sodium chloride solution supplied to the cut end.

Graph 19 B

Shoot 10 ins. long, mature; 6 pairs of leaves; stem bare up to a height of 5 ins. from the cut end.

Weather: Dull till about 9-30 a.m. and then bright; warm. T: 89°F. at 10-30 a.m.

Shoot cut at 7-30 a.m., and recording from 8 a.m. Rate of transpiration slow till 9-30, weather being dull. Rate at 10-20 a.m. ($2\frac{1}{2}$ M.).

Supplied 10 per cent. sodium chloride solution at 10-20 a.m. Rate steady till 1 p.m. ($2\frac{1}{2}$ M.), with a slight increase between 12 noon and 12-30 p.m. Rate slowly going down from 1 p.m. Lower leaves drooping down by 1-20 p.m. Rate at 2 p.m. ($3\frac{1}{4}$ M.). Lower leaves completely drooping and discoloured along the margins near the tips. Rate at 4 p.m. ($3\frac{1}{2}$ M.). Upper leaves, only flaccid.

In this experiment, the fall in the rate of transpiration starts sometime after supplying the cut end with 10 per cent. Sodium chloride solution. As the leaves are seen only at a height of 5 ins. from the cut end, it is no surprise to notice the fall in the rate starting so late. The fall starts when the solution reaches the leaves. In this experiment, the shoot used is quite mature and thus the fall in transpiration is very little compared to that in the previous experiment.

Experiment No. 20.—5th July 1932.

5% sodium chloride solution supplied to the cut end.

Graph 20

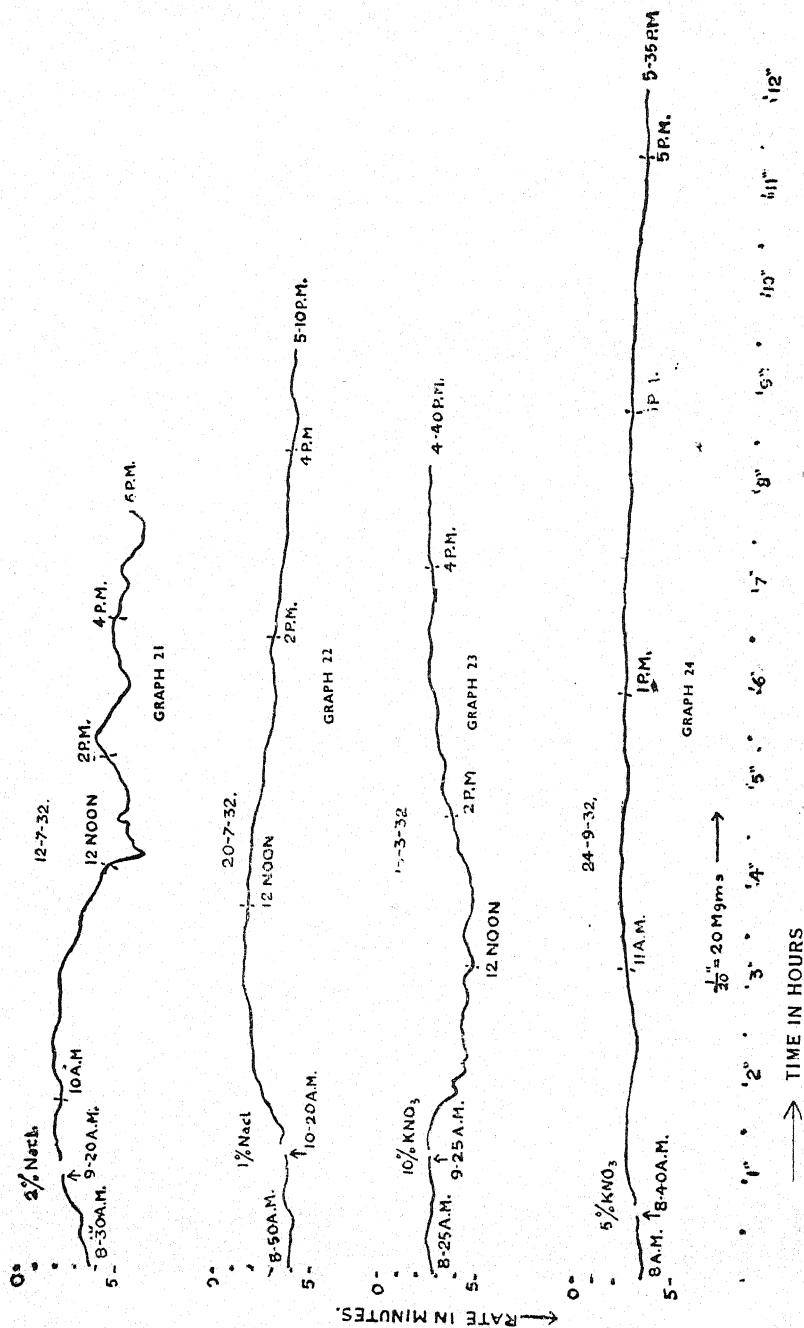
Shoot: 1 ft. long; young; 4 pairs of leaves; long internodes.

Weather: Bright; warm. T: 86°F. at 9-30 a.m.

Shoot cut at 7-15 a.m. and recording from 8-15 a.m. Rate steady from 9 a.m. ($2\frac{1}{4}$ M.).

Supplied 5 per cent. sodium chloride solution at 9-17 a.m. Rate going down very slowly from 9-40 a.m. Rate at 10 a.m. (3 M.). A slight increase in the rate between 10 a.m. and 11 a.m. Slow fall in the rate again from 11 a.m. Lower leaves completely drooping by 11-45 a.m. Drooping of the upper leaves not clear. Steady rate of $4\frac{1}{2}$ M. between 12 noon and 3 p.m. and again a slow fall. Rate at 5 p.m. ($6\frac{1}{4}$ M.). Discoloration in the lowest leaves spreading inwards from the margins. Uppermost leaves showing signs of flaccidity.

The fall in the rate started quite soon as the solution could reach the first pair early. The fall was not continuous but the rate was getting steady in 2 places though of course the rate was going down in the end. These steady rates might denote the time intervals for the solution to reach the different pairs of leaves inserted at different levels, especially as the internodes were long.



Experiment No. 21.—12th July 1932.

2% sodium chloride solution supplied to the cut end.

Graph 21

Shoot: 8 ins. long; young; about 4 pairs of large fully turgid leaves.

Weather: Cool; fairly bright. T: 87°F. at 1 p.m.

Shoot cut at 7-30 a.m. and recording from 8-30 a.m. Rate slow to start with but rapidly increasing. At 9 a.m. ($3\frac{1}{4}$ M.).

Supplied 2 per cent. sodium chloride solution at 9-20 a.m. ($2\frac{1}{2}$ M.). No fall in the rate till about 11 a.m. A fairly rapid fall till 12-30 p.m. ($5\frac{1}{2}$ M.). Afterwards, rate oscillating till the end of the experiment. Neither drooping of the leaves nor any discoloration in the leaves.

With 2 per cent. sodium chloride solution also, a fall in the rate was obtained after sometime, though the rate was afterwards fairly steady within small limits till the end of the experiment. Though there was a fall in the rate, there was no drooping of the leaves.

Experiment No. 22.—20th July 1932.

1% Sodium chloride solution supplied to the cut end.

Graph 22

Shoot: 10 ins. long; mature; leaves from a height of 5 ins. from the cut end; about 10 small leaves.

Weather: Bright; warm. T: 89°F. at 11-30 a.m.

Shoot cut at 7-30 a.m., and recording from 8-50 a.m. Rate rather slow to start with but gradually going up from about 9-30 a.m.

Supplied 1 per cent. sodium chloride solution at 10-20 a.m. ($3\frac{3}{4}$ M.). Rate increasing till 12 noon as would have happened normally with the cut end in water. Rate at 11 a.m. ($2\frac{1}{2}$ M.) and at 12 noon ($1\frac{1}{2}$ M.). Then a slow fall in the rate. Experiment stopped at 5-10 p.m. (4 M.). Neither drooping of the leaves nor any discoloration.

1 per cent. sodium chloride solution appears to have very little effect on transpiration during the experiment. A continuous supply of even 1 per cent. solution to the cut end brings about an accumulation of the salt in the leaves, thus acting as a hypertonic solution. This is shown in the case of absorption with a supply of 1 per cent. Sodium chloride solution to the cut end (Experiment 10); the rate of absorption does not recover next morning as it would have done

if the cut end is in water throughout the experiment. If the experiment on transpiration was continued till next morning, the effect of such accumulation on transpiration would have been noticed.

Effect of a supply of different concentrations of sodium chloride solution to the cut end on transpiration.

In all concentrations of the sodium chloride solution when supplied to the cut end of a shoot, there is no fall in the rate of transpiration as the solution is passing up the stem.

When the solution enters the leaves, the fall starts, the magnitude and rate of the fall depending on the concentration of the solution and the age and the water-content of the shoot. With higher concentrations supplied to the cut end of a young shoot, there is a rapid big fall in the rate as the solution enters the leaves. With lower concentrations supplied to such a shoot, the fall is relatively less. In old shoots even with the higher concentrations, the fall is much less.

When the solution has entered all the leaves, the fall gets slowed down and in the lower concentrations, the lower rate may keep more or less steady.

The effect of supplying potassium nitrate solutions to the cut end on transpiration is more interesting.

Experiment No. 23.—16th March 1932.

10% potassium nitrate solution supplied to the cut end.

Graph 23

Shoot: 10 ins. long; 7 pairs of leaves; not mature.

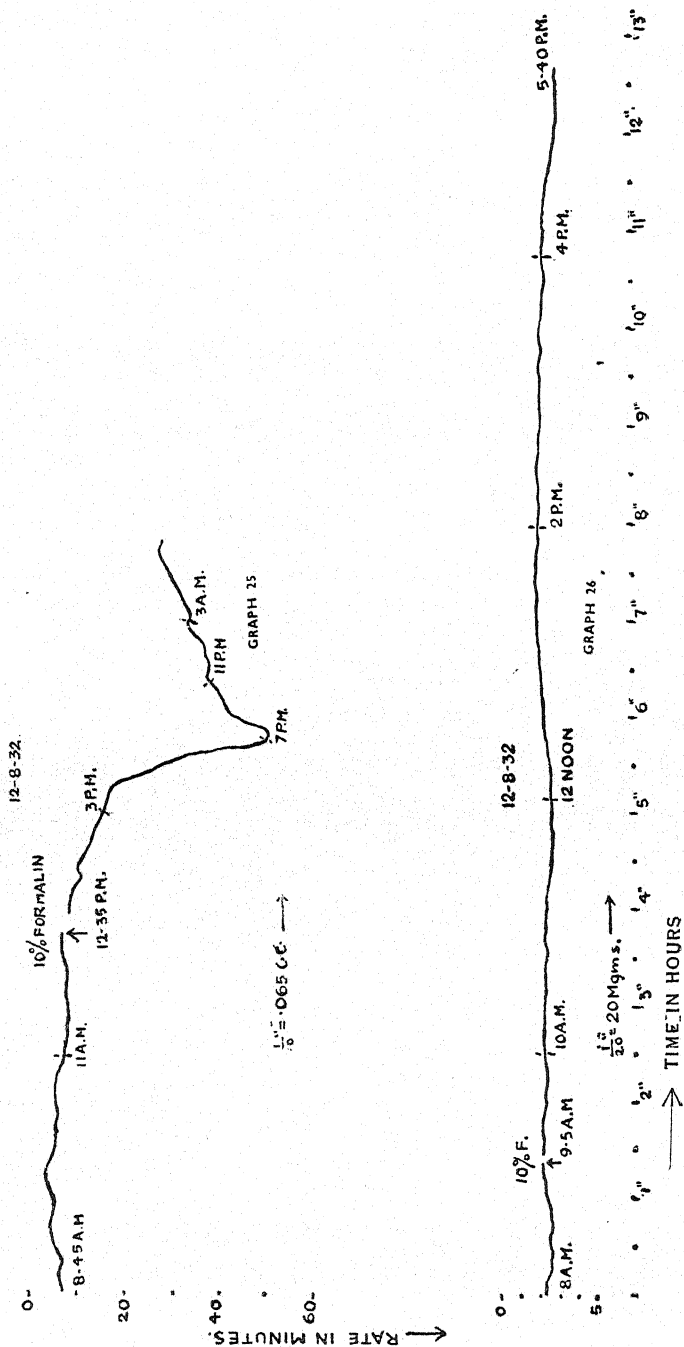
Weather: Bright; warm. T: 84°F. at 11 a.m.

Shoot cut at 8 a.m. and recording from 8-25 a.m. Rate steady (3 M.).

Supplied 10 per cent. potassium nitrate solution at 9-25 a.m. Rate constant till 10 a.m. Then a fairly rapid fall in the rate. Minimum rate at 10-45 a.m. (4½ M.). This slow rate continued with small variations till 1 p.m. Then an increase in the rate. Recovery complete by 3-15 p.m. (2 M. *i.e.*, quicker than the initial rate). This rapid rate kept on till the end of the experiment.

Visible drooping of the lower leaves by 12-30 p.m. Lower leaves well discoloured in patches by 4-30 p.m. Upper leaves flaccid.

With 10 per cent. potassium nitrate solution supplied to the cut end, there is a fall in transpiration as the solution reaches the leaves. But instead of this fall being continuous as it is in the case of sodium chloride solution it stops soon, and the rate gets steady for sometime. Then the rate slowly begins to recover. Discoloration



of some of the leaves in patches is noticed at this stage. The recovered rate is slightly higher than the initial rate and keeps constant for some hours.

Experiment No. 24.—24th September 1932.

5% potassium nitrate solution supplied to the cut end.

Graph 24

Shoot: 1 ft. long; mature; stem bare up to a height of 7 ins. from the cut end; a few small branches; about 20 small leaves.

Weather: Warm; bright.

Shoot cut 7-30 a.m. and recording started at 8 a.m. Rate more or less constant ($3\frac{1}{2}$ M.).

Supplied 5 per cent. potassium nitrate solution at 8-40 a.m. A slow increase in the rate. At 9-30 a.m., it was ($2\frac{1}{2}$ M.). A fairly constant rate from 11-30 a.m. till 3 p.m. (2 M.). Then a very slow fall till 5 p.m. ($2\frac{3}{4}$ M.).

Leaves slightly flaccid at about 11 a.m.

With 5 per cent. potassium nitrate solution supplied to the cut end, the rate of transpiration did not show much variation. There appeared to be a fall at 10-30 a.m., but was not clear. The rate later on was a little higher than the initial rate and was fairly constant. Drooping of the leaves was not clear and there was no discoloration of the leaves.

Relation of Water-content to Formalin Supply

The following two experiments, showing the effect of 10 per cent formalin solution on the rate of absorption and rate of transpiration, give us definite evidence as to how this effect is influenced by the water-content of the shoot.

Till a day previous to that on which the shoots were cut, the weather was bright and warm. The soil on which the plant was growing was dry. The same evening the skies were dark with clouds and it rained heavily during the night. The next morning, the plant appeared fresh and shoots were cut at 7-40 a.m.

Experiment No. 25.—12th August 1932.

Effect on *absorption* of supplying 10 per cent. formalin to the cut end of a *young and turgid shoot*.

Graph 25

Shoot: 1 foot long; young and well-growing; stem bare up to a height of 5 ins. from the cut end; small branches at the lower axils; about 20 leaves.

Weather: Warm; bright. T: 88°F. at 2-30 p.m.

Recording from 8-45 a.m. Rate of absorption slightly rapid between 9-30 a.m. and 10-30 a.m. Then it was steady. Rate at 12-30 p.m., 6 M. for absorbing .065 cc.

Supplied 10 per cent. formalin at 12-35 p.m. Rate started going down almost immediately though rather slowly till 2 p.m. (12½ M.). Then a little more rapidly till 4 p.m. (20 M.). From 4 p.m., the curve was very steep. Minimum rate at 7 p.m. (51 M.). Then a rapid recovery till 9 p.m. (42 M.). Rate of recovery slowed down but recovery continued till next morning. Rate at 7 a.m. next morning, (28 M.).

No signs of discoloration in any of the leaves even by 2-30 p.m. By 4-30 p.m., discoloration was clear in all the leaves.

The fall in the rate of absorption starts immediately after supplying the cut end with 10 per cent. formalin. From 4 p.m., there is a big fall in the rate. At about this time, the discoloration in the leaves is also seen. The recovery in the beginning is fairly rapid but afterwards it slows down. The recovered rate is only one-fourth of the initial rate.

When this experiment is compared with the one (Experiment 4) already described, a clear difference is noticeable in the magnitude of the fall. But the curves in their general course do resemble each other. The difference is evidently due to the difference in the condition of the shoot, in the two cases.

Experiment No. 26.—12th August 1932.

Effect of supplying 10 per cent. formalin to the cut end
of a *young and turgid shoot on transpiration.*

Graph 26

Shoot: 10 ins. long; stem bare up to a height of 5 ins. from the cut end; a few small branches; about 15 leaves; young and well-growing.

Recording from 8 a.m. Rate of transpiration steady. At 9 a.m. it was (2 M.). Supplied 10 per cent. formalin at 9-5 a.m. No fall in the rate till 4 p.m., any variation being only about half a minute on either side. Rate going down slowly from 4 p.m. At 5-40 p.m., it was (3 M.).

Slight discoloration in all the leaves along the veins by about 11-30 a.m. By 4-30 p.m., all the leaves were well discoloured.

There is a clear difference between the curve obtained for this experiment and the one obtained with the same treatment for Experiment 16. In the latter there is a clear fall and a rise in the rate of transpiration after supplying the cut end with 10 per cent.

formalin. But in the experiment described above, the fall is not seen. The cause for the absence of this fall appears to depend on the condition of the shoot.

Interpretation of the Results

ABSORPTION.

When a solution of either a non-permeable, permeable or permeable and toxic substance is supplied to the cut end of a shoot, the course of the subsequent rate of absorption can be divided into 3 main stages:

- (i) when the solution is passing up the stem,
- (ii) as it enters the leaves, and
- (iii) after its entry into the leaves.

The effect on the rate of absorption during these stages depends on the nature of the substance, its concentration in the solution supplied to the cut end, the age and the water-content of the shoot.

When any of the different solutions used were supplied to the cut end, the effect observed at the first stage was a fall in the rate of absorption. It may be due to two causes: (1) A blocking of the vessels by a precipitate in their lumen, and (2) to osmotic extraction of water from the stem tissue into the vessels. The differences in the magnitude of the fall when different concentrations of the same substance were supplied, indicate that the latter is the cause for the fall in the rate of absorption. Microscopical examination has shown that no precipitate is formed in the lumen. The water withdrawn osmotically into the vessels reduces the rate of absorption. The amount of water withdrawn from the stem tissue and the rate of withdrawal depend on the concentration of the solution supplied to the cut end and the age and the water-content of the shoot. Thus the fall in absorption is quite high with young and turgid shoots and higher concentrations of the solution. With older shoots and low water-content and even with the higher concentrations of the solution, the fall in absorption is much less, as the amount of water extracted from the stem tissue in such shoots is less. With lower concentrations of the solution using young and turgid shoots, there is a little amount of water osmotically withdrawn from the stem tissue but with older shoots, this is quite negligible.

All the three substances in their different concentrations bring on similar effects in the first stage.

II. As the solution enters the leaves, in the case of all the three substances, there is, to start with, a quicker fall in absorption, the rate and magnitude of the fall depending in the same way as described previously on the concentration of the solution and the age and the water-content of the shoot. This further depression

in the rate of absorption is to be attributed to the greater quantity of water that is withdrawn from the leaf-cells as the solution reaches them.

This similarity in the effect of the different substances used extends only to the first part of the curve at this stage. Very soon differences are noted in the further course of the curve due to the properties of the substances used in solution.

Apart from the differences due to the nature of the shoots themselves, the specific effects of the different substances are:

With sodium chloride solution and with formalin, this fall in absorption is continuous. With potassium nitrate solution the rate tends to become steady at a lower level after a time. Though the observed effect is more or less similar in the first two cases, the causes are not the same.

With sodium chloride solution, the further rapid fall in absorption in the second stage is mainly due to the osmotic effect of the solution on the leaf-cells.

With potassium nitrate solution, this fall does not continue for long. Along with the osmotic withdrawal of water from the leaf-cells there is a simultaneous entry of the salt into the cells as the salt itself is permeable. An entry of the salt into the leaf-cells increases the concentration of the cell-sap which results in reducing the withdrawal of water from these cells by the solution in the vessels. Thus there is a tendency for the rate of absorption to get steady at this stage.

In the case of formalin solution, which is known to be a rapidly penetrating as well as a highly toxic substance, along with the osmotic withdrawal of water from the leaf-cells, there is a simultaneous entry of the formalin into the cells, just as in the case of potassium nitrate. But with the formalin, an accumulation of the substance even in very small quantities inside the cells kills them. So there is no possibility in this case of an increase in the osmotic value of the cell-sap of the living cell. But even before the osmotic effect of the formalin solution on the leaf-cells can be complete, these cells are killed. With the death of these cells, their permeability is altered and the cell-sap is released to the outside due to the recoil of the cell-wall. This release of the cell-sap brings about a reduction in the transpiration pull which causes a fall in absorption. The magnitude and rapidity of this fall depend again on the concentration of the formalin supplied to the cut end, the age and the water-content of the shoot. The fall in absorption is quite considerable if the amount of cell-sap released from the leaf-cells during their death is large. This depends on the water-content of the leaf-cells and their wall-pressure at the time they are killed. If the wall-pressure is big and the water-content is also

high, when these cells are killed, the amount of cell-sap that is pressed out will be large and the consequent reduction in transpiration pull high. This is what happens when 10 per cent. formalin solution is supplied to the cut end of a young turgid shoot. The osmotic effect of the solution on the leaf-cells is little but the concentration of the solution is enough to bring about the death of these cells fairly rapidly. Thus at the time of their death, their large water-content and the high wall-pressure are not altered. The amount of cell-sap sent out by expulsion due to the recoil of the highly stretched cell-wall is large and thus a big fall in absorption is noticed. But with 50 per cent. formalin at the cut end of a similar young and turgid shoot, though there is a similar big fall in absorption as the solution enters the leaves, the causes for the same are not similar to those mentioned in the case of 10 per cent. formalin. With a supply of the higher concentration of formalin to the cut end, there is a greater osmotic withdrawal of water from the leaf-cells before these are killed. This brings about a fall in absorption. Now due to the osmotic withdrawal of water from the leaf-cells, before they are killed, the water-content as well as the wall-pressure of the cells are lowered. Therefore, with the death of the leaf-cells, there is less expulsion of the sap from these cells. But with 50 per cent. formalin, the total fall in absorption due to the osmotic withdrawal of water from the leaf-cells and the release of cell-sap from these cells during their death, will be more or less equal to the fall in absorption with 10 per cent. formalin which is mostly due to the expulsion of cell-sap. This difference in the action of the 10 per cent. and 50 per cent. formalin concentrations is not evident from the absorption curve at this stage, because the net effect on absorption is the same in whatever manner the excess water is obtained. The effects of this difference in the *modus operandi* of the 10 per cent. and 50 per cent. concentrations will be seen in the third stage, and also in transpiration.

With old shoots of low water-content, there is not much of expulsion of sap with the killing of the leaf-cells as the cell-walls are fairly rigid; also as the cell-sap is of a higher concentration, there is less amount of water withdrawn osmotically even when the shoot is supplied with 50 per cent. formalin. Thus though a high concentration such as 50 per cent. formalin is supplied to the cut end of an old shoot of low water-content, the fall in absorption with the death of the leaf-cells is very little.

The rate of this fall in absorption during the second stage is quite high with young turgid shoots in higher concentrations of formalin and a little less in the lower concentrations. In higher concentrations, the osmotic withdrawal of water from the leaf-cells as well as their killing are very rapid. In lower concentration such as 10 per cent. formalin though the osmotic effect is slow, the killing effect is fairly rapid as the critical concentration needed to bring about the death of the cells is low, and with the death of the cells,

there is a greater expulsion of the sap as the wall-pressure is higher. With older shoots, the rate of fall in absorption is proportional to the concentration of formalin supplied.

III. The third stage of the absorption curve is characterised by marked difference with the use of the three substances.

With a continuous supply of the sodium chloride solution, after it has entered all the leaves, the fall in absorption still continues though at a lower rate than in the previous stage. As water is being extracted from the leaf-cells by the solution on its entry into the leaves, the cell-sap of the leaf-cells will be gradually getting more and more concentrated and there will be less extraction of water from them. Thus the fall in absorption at this stage is not so rapid as it is in the previous stage but continues at a lower rate.

In the case of the permeable salt, potassium nitrate, during the second stage itself along with the extraction of water from the leaf-cells, there is a simultaneous entry of the salt into the cells which will be reducing the osmotic withdrawal of water from these cells and make the rate of absorption go down at a very slow rate. Now with a continuous supply of the solution at the cut end, the salt gradually gets accumulated in the leaf-cells and when it passes a certain critical concentration kills the cells. This again brings about a release of the cell-sap from these cells. But this process is slow and extends over a long period so that the fall in absorption due to the release of cell-sap from the cells during their death, is distributed over a long period and a very slow fall in absorption is noticed during this period. With the lower concentration, the accumulation of the salt itself in the leaf-cells is much more gradual and the death of these cells due to accumulation of the salt is also delayed very much. Thus after the solution has entered the leaves, there is a very gradual slow fall in absorption.

In the case of the formalin solution, a completely different course is followed during this third stage. When the cells in most of the leaves are killed, an increase in the rate of absorption is observed. The level finally reached depends on the concentration of the formalin used and the age and the water-content of the leaf-cells. The rate may recover to an extent equivalent to the initial rate or may go up only to half the initial rate.

A recovery in the rate of absorption can be brought about only by a re-establishment of suction from above, *i.e.*, the transpiration pull. When the cell-sap was released from the leaf-cells by their death, this liquid should have reduced the transpiration pull and depressed the rate of absorption. But very soon evaporation will overtake the excess sap supply and will tend to re-establish the transpiration pull and an increase in the rate of absorption will result.

But the feature of interest is that the re-established transpiration pull can be as big as that in the living leaf. Such a result implies that the mechanism for transpiration and suction is little disturbed by the death of the cells in the leaf, at least for a fairly long period after their death.

The differences in the magnitude of recovery in the rate of absorption will be clear from the following considerations. The amount of cell-sap released with the death of the cell depends on the recoil of the cell-wall and in no case does it bring out all the cell-sap from the interior of the cell. The cell-sap that is remaining in the dead cells is still capable of enabling the cell to absorb water from the vessels. This can be possible only if the semi-permeable nature of the leaf-cell is not completely destroyed. A recovery in the rate of absorption to about half the initial rate after the leaf-cells are killed shows that the dead cell is still capable of behaving osmotically active to a certain extent thus bringing about a re-establishment of the transpiration pull.

The magnitude of the recovered rate depends on the amount of cell-sap remaining inside the dead cell. So if a cell is killed when its wall pressure is high, the amount of cell-sap remaining inside the cell is comparatively less. In a young and turgid shoot, the wall pressure is quite high. When 10 per cent. formalin is supplied to its cut end, after the death of the leaf-cells, the recovered rate is far less than half the initial rate. But if 50 per cent. formalin is supplied to the cut end of a similar young and turgid shoot, before the death of the leaf-cells their wall-pressure is reduced to a certain extent by the osmotic withdrawal of water from these cells; thus the amount of cell-sap released is less than in the previous case and consequently the recovered rate is a little higher. In old shoots, with low water-content, the difference in wall-pressure in maximum and minimum turgor is very little. Thus with the death of the leaf-cells there is less of cell-sap released and so the recovered rate is even equal to the initial rate.

The rate of recovery in absorption depends on the concentration of formalin supplied to the cut end and the weather conditions. With higher concentrations the rate of absorption recovers soon, if the weather is warm and dry, because the leaf-cells are killed rapidly and the evaporation of the released sap is also rapid. In lower concentration, the killing is comparatively slow and so also is the recovery.

The period for which the recovered rate keeps constant depends again on the behaviour of the dead-cell. The first effect of killing is a release of the cell-sap, its extent depending on the wall-pressure. The later effect is a slow exosmosis of substances from inside the dead cell. With the death of the cell, the cell is not made thoroughly permeable to all the solutes inside the cell. Also the

different solutes do not pass out at the same rate. These factors allow the cell to be osmotically active but after a complete equilibrium is set up between the inside and outside of the cell, the cell can no longer be osmotically active. This complete equilibrium is not possible with a transpiring cell where evaporation is going on at one end of the cell increasing the concentration of the cell-sap. But due to higher evaporation from the dead leaves than what can be replaced by the liquid that is absorbed at the cut end there is a curling of the leaves, which reduces the evaporating surface thus bringing about a slow fall in absorption.

In young and turgid shoots, when a higher concentration of formalin is supplied to the cut end, death of the leaf-cells occurs after a certain amount of water is osmotically withdrawn by the formalin. So the wall-pressure is reduced and the release of the cell-sap is not to the same extent as it is when a lower concentration is supplied to the cut end. Thus in the latter case the recovered rate begins to go down a little earlier than in the former. In both cases the time for which the recovered rate keeps constant depends also on the condition of the shoot after death. With the higher concentration, the stem gets shrunken as the solution passes up the stem and the shoot collapses at the nodes. These factors do not allow the vessels to be maintained in their original normal condition. The vessels get compressed and thus the absorption goes down. In the lower concentration with the same young and turgid shoots, the shrinkage in the stem tissue as the solution passes up the stem is much less and the shoot does not collapse for a long time. In old shoots of low water-content, the recoil of the cell-wall is not high with the death of the cell. Thus with such shoots, in all concentrations, the recovered rate keeps fairly constant for a long time. This is also aided by the absence of a collapse of the shoot and also by only a slight shrinkage in the stem.

Transpiration

In the same way as described for absorption, in the case of all the three substances, the course for the rate of transpiration can also be divided into 3 stages from the time of supplying the solution to the cut end:

- (i) while the solution is passing up the stem,
- (ii) as the solution enters the leaves, and
- (iii) after the solution has entered all the leaves.

I. With all the three substances, *i.e.*, sodium chloride, potassium nitrate and formalin, in solution, the osmotic withdrawal of water from the stem tissue, as the solution is passing up the stem lowers the rate of absorption but it does not affect the transpiration. Until the solution reaches the leaves, there is no change in the rate of transpiration.

II. With all the three substances, when the solution enters the leaves, water from the leaf-cells is withdrawn osmotically. This results in a gradual increase in the concentration of the cell-sap which brings about a fall in transpiration. The magnitude and rate of fall in all the three cases again depend on the concentration of the solution, the age and the water-content of the shoot. With young and turgid shoots, and higher concentration of the solution, the fall in transpiration is quite rapid and also big. With lower concentrations using similar young and turgid shoots, the fall in transpiration is relatively slow and also much lower in magnitude as the withdrawal of water from the leaf cells is less rapid and less amount of water is withdrawn. With older shoots even using higher concentrations of the solution, the fall in transpiration is little as very little of water is withdrawn osmotically from the leaf-cells.

With sodium chloride solution at the cut end, the rate of transpiration continues to go down as the solution enters all the leaves, for there is a continuous withdrawal of water in all the leaves. But with 10 per cent. potassium nitrate solution at the cut end, the fall in transpiration is not continuous. As has been already shown in the explanation of the results on absorption, there is a simultaneous entry of the salt into the leaf-cells along with the osmotic withdrawal of water from these cells by the solution in the vessels. This entry of the salt into the leaf-cells reduces further withdrawal of water from them. Thus the fall in transpiration gradually stops and a more or less steady rate of transpiration is obtained. At this stage, the rate of absorption also tends to get steady. With the lower concentration, *i.e.*, 5 per cent. of the potassium nitrate solution at the cut end, the fall itself is very little, the rate keeping fairly steady even when the solution is entering the leaves.

With a supply of formalin solution to the cut end, the fall in transpiration noticed as the solution enters the leaves, has been already explained as due to the osmotic withdrawal of water from the leaf-cells. But this fall does not continue for long. Along with the withdrawal of water from the leaf-cells, there is a simultaneous entry of the formalin itself into the leaf cells, as it is in the case of potassium nitrate. But in the case of formalin, an accumulation of the substance inside the cells, kills them. Thus the stage of a steady rate of transpiration, before the leaf-cells are killed, as seen in the case of potassium nitrate, is absent here. But with the death of the leaf-cells, there is an expulsion of the cell-sap which immediately increases transpiration. Thus before the osmotic effect of the formalin solution on the leaf-cells is complete, these cells are killed resulting in a recovery in the rate of transpiration. This recovered rate is either equal to the initial rate or even higher, depending again on the concentration of the solution supplied and

the age and the water-content of the shoot. With young and turgid shoots and a higher concentration of formalin, the fall in transpiration is quite evident as the water in the leaf-cells is withdrawn osmotically by the formalin solution. The recovered rate with the death of the leaf-cell does not go in the beginning beyond the initial rate, as the water-content of the leaf-cells as well as their wall-pressure are reduced before these cells are killed. But with a lower concentration using very young and turgid shoots, fall in transpiration is insignificant as the osmotic withdrawal of water is very little and the death of the cells results in an expulsion of the cell-sap in large quantities. The latter brings about a higher rate of transpiration thus masking the osmotic effect of the formalin solution on the leaf cells. The recovered rate in this case in the beginning is even higher than the initial rate. In mature, turgid, and old shoots, in all concentrations, the recovered rate is more or less equal to the initial rate. •

Thus when formalin is supplied to the cut end, the fall in the rate of transpiration is due to the osmotic withdrawal of water from the leaf-cells and the subsequent rise in transpiration is due to the release of the cell-sap due to the death of the cells.

III. During the third stage again, the course of transpiration followed in the three cases is different. With sodium chloride solution, the fall in transpiration observed in the second stage gets less rapid. This is because the cell-sap in the leaf-cells gets more and more concentrated due to extraction of water from them, thus reducing further osmotic withdrawal of water from these cells.

With potassium nitrate solution, the accumulation of the salt inside the leaf-cells, which started in the second stage, continues and when it exceeds a certain critical concentration, the cells are killed. This results in a release of the cell-sap from these cells, which brings about an increased rate of transpiration. This process of killing the cells is very slow in this case and so the recovery in the rate is also slow. That the cells are being killed at this stage is evident from the patches of discoloration in the leaves.

The recovered rate is equal to the initial rate and it keeps constant for a fairly long time. At the beginning, this recovered rate is kept on due to the evaporation of the released sap from the killed cells but the later constant rate is no doubt due to the capacity of the dead cells in the leaf to absorb water and make it available for evaporation.

In the case of formalin solution, in the third stage of the curve for transpiration, the recovered rate, observed in the second stage, keeps fairly constant for a long time. The period, for which it keeps constant, depends again on the age and the water-content of the shoot as well as the concentration of formalin supplied to the cut end. The recovered rate keeps constant for sometime because

the dead cells are able to behave more or less normally as regards the mechanism for transpiration. They are able to absorb water from the vessels and make it available for evaporation. This capacity of the dead cells to absorb water depends on the amount of cell-sap remaining in the cells after the expulsion of some of the cell-sap which is brought about by the recoil of the cell-walls during the death of these cells. Thus with young and turgid shoots in a lower concentration of formalin, the recovered rate does not keep constant for a long time, while with the same shoot in a higher concentration, the recovered rate keeps constant for a little longer than in the previous case. Here the amount of released cell-sap is less than that obtained with the lower concentration. But with the higher concentration also in most cases the recovered rate does not keep constant for a long time as a normal supply of the solution is hindered by the mechanical disturbance of the vessels in the stem brought about by a big shrinkage in the stem tissue. The shrinkage in the stem tissue and the consequent disturbance of the conducting paths is not seen to the same extent in the lower concentration of formalin. So taking these points into consideration, the difference in the periods in the two cases for which the recovered rate keeps fairly constant is not often quite evident though the causes for this result are not the same in the different treatments.

With older shoots in all concentrations, the recovered rate keeps fairly constant for a long time for here the expulsion of the cell-sap with the death of the leaf-cells is small, the shrinkage in the stem tissue is much less, and also the shoot does not collapse.

Relation between Absorption and Transpiration Curves

A comparison of the absorption and transpiration curves will give us a better insight into the effects, on these two processes, of the different concentrations of the three substances in solution when supplied to the cut end.

With a supply of a hypertonic solution of either sodium chloride, potassium nitrate or formalin, there is an initial fall in absorption as the solution is passing up the stem due to the osmotic withdrawal of water from the stem tissue. The magnitude of the fall depends on the concentration of the solution supplied, the age and the water-content of the shoot. This osmotic extraction of water from the stem tissue does not affect the rate of transpiration except in very young and turgid shoots with a supply of a high concentration of sodium chloride or formalin. This is due to a reduction in water-supply caused by the mechanical constriction of the vessels which is brought about by a shrinkage of the stem tissue.

An extraction of water from the leaf-cells by the osmotic solution results in a flow of water into the vessels, affecting rate of

absorption, and also in an increase in the concentration of the cell-sap of these cells. This increase in the concentration brings about a fall in transpiration and thus a lowering of the transpiration pull which causes a fall in the rate of absorption. So, when the solution enters the leaves, the fall in absorption noticed in the previous stage continues at a more rapid rate and a fall in transpiration is brought about for the first time and this feature is common to all the three substances.

The further course in the absorption and transpiration curves is affected by the nature of the substance, whether it is non-permeable, permeable, or permeable and toxic. In the case of the non-permeable salt, sodium chloride, the fall in both absorption and transpiration noticed in the previous stage, as the solution enters the leaves, continues at more or less the same rate as the solution spreads in the leaf-tissue.

With the permeable salt, potassium nitrate, the fall in absorption as well as transpiration, noticed as the solution enters the leaves, begins to slow down when the solution spreads into all the leaves. This is brought about by an increase in the osmotic value of the cell-sap in the leaf-cells. This increase is caused partly by a withdrawal of water from these leaf-cells by the osmotic solution but mainly by a diffusion of the salt itself into these cells. This increase in the concentration is further accelerated by the evaporation of water from these cells. Thus after reaching a certain limit, a flow of water is made possible from the solution in the vessels into the transpiring cells and from them to the outside. An equilibrium is thus set up between the transpiration and absorption at this lower level and the curves for transpiration and absorption keep more or less steady at this stage and run parallel.

In the case of formalin, which is also permeable, before this steady rate in absorption and transpiration, as noticed in the potassium nitrate solution, could be obtained, the cells themselves are killed due to the entry of the highly poisonous formalin into them. Before the osmotic effect of the formation on the leaf-cells in all the leaves is complete, the death of some of the cells occurs which results in a release of the cell-sap. This brings about a fall in absorption by a reduction of the transpiration pull but an increased rate in transpiration owing to the availability of more water for evaporation. With young and turgid shoots, with a supply of a low concentration of formalin, the magnitude of the fall in absorption with the death of the leaf-cells is considerable. While a fall in transpiration occurs in some cells due to the osmotic withdrawal of water from them, others are releasing cell-sap due to death. The former effect is masked by the latter and the net result is an increase in transpiration.

During the third stage of the curves on absorption and transpiration, *i.e.*, after the solution has spread into all the leaves, a still further difference is seen according to the nature of the substance. With a supply of the non-permeable salt in solution, the fall in absorption as well as in transpiration begin to slow down. A continuous withdrawal of water from these cells brings about a gradual increase in the concentration of cell-sap in these cells. Beyond a certain limit, the osmotic extraction of water from them is stopped and a reversal in the flow of water takes place. Water is extracted by these cells from the solution in the vessels and is evaporated to the outside. Thus an equilibrium, as was noticed in the case of potassium nitrate, is made possible for absorption and transpiration and thus the curves in the two cases keep fairly steady and run parallel.

With a supply of the permeable salt in solution to the cut end, the salt continues to get accumulated in the leaf-cells. This accumulation of the salt beyond a critical concentration proves toxic to the cells and results in the death of these cells. This brings about again a release of the cell-sap from the cells which means a fall in absorption and a recovery in the rate of transpiration.

In the case of formalin, with the death of the leaf-cells, the curves for absorption and transpiration run fairly parallel and are quite steady for a long time. The rates in both the cases tend to keep up to the initial rate. This could be made possible only if the mechanism for transpiration and absorption has not been destroyed with the death of the leaves.

Conditions of the Shoots with the Three Treatments

1. *Wilting and collapse of the shoot*

With sodium chloride and formalin solutions at the cut end, the changes in the condition of the shoot appear to be similar in the two cases. In very young and turgid shoots, a higher concentration of either solution brings about a drooping of the shoot tip even before the solution reaches that region. This drooping of the shoot tip is no doubt the result of a reduction in water-supply brought about by an increased resistance to the passage of sap through the vessels. It may be caused in one of two ways: by deposits inside the vessels which block the lumen, or by a mere mechanical constriction of the vessels. There may be put forward another cause and perhaps the sole one, according to Sir J. C. Bose, that the "pumping cells" in the stem are killed resulting in a reduction of water-supply. But sufficient proof has already been given in a previous paper that this argument is groundless. Now turning our attention to the blocking of the vessels, it has already been mentioned that microscopical examination of the vessels had not revealed any internal deposits. Further, such a blocking should bring about

wilting of the tips in all the different kinds of shoots used and in other concentrations. But this result is obtained only with young turgid shoots and higher concentrations. So blocking of the lumen by deposits is ruled out. On the other hand there is enough evidence to show that a large shrinkage of the stem due to the osmotic withdrawal of water, as the solution is passing up the stem, occurs. This reduction in the stem size naturally does not allow the vessels to be supported in their original condition for a normal conduction of the sap in them. This results in a reduction in water-supply to the shoot tip and brings about its drooping.

In both the solutions again in higher concentrations, as the solution passes into the leaves, these leaves begin to droop. This is very clear in young and turgid shoots, but it may be absent in the older shoots with low water-content, even with the higher concentrations. This drooping of the leaves is due to the osmotic withdrawal of water from the petioles and also from the leaves resulting in a flaccidity of the leaf and a slow drooping. In the older shoots, the osmotic extraction of water is much less and also the change in size in the tissue under maximum and minimum turgor is very little. Thus in such shoots, drooping does not result. With lower concentrations of either solution even with young shoots, the drooping is not clear as the osmotic values of these solutions are low.

With potassium nitrate solution also, with young and turgid shoots and a higher concentration (10 per cent.), the drooping of the leaves is quite clear. But with the lower concentration (5 per cent.), it is not clear.

Finally the phenomenon of the collapse of the shoot at the nodes usually near the top portion of the shoot will be considered. With the higher concentration of either sodium chloride solution or formalin solution with young and turgid shoots, after the solution has passed through the whole stem, the shoot begins to collapse at the top nodes. This is due to a weakening of these parts (which are swollen in this plant) mainly by the osmotic withdrawal of water from the nodal tissue in the case of sodium chloride solution. But with a supply of the formalin solution to the cut end, the loss of turgidity in these tissues is caused by both the osmotic and the killing effect of the formalin on the nodal cells. This results in a collapse of the shoot in these regions, *i.e.*, the shoot bends down at these nodes and hangs loose. With lower concentrations of sodium chloride solution, with similar young and turgid shoots, the collapse of the shoot does not occur. But in the case of formalin even with the lower concentrations with such young shoots, there is a collapse of the shoot at the nodes but this occurs much later. The nodal cells get killed by the formalin. These cells are fully turgid at the time of their death, as the osmotic withdrawal of water from these cells by these low concentrations of formalin is

very little. The cell sap is forced out by the recoil of the cell-walls when the cells are killed and the cells lose their mechanical rigidity. With older shoots in any concentration of either solution there is no collapse of the shoot.

With potassium nitrate solution, the collapse of the shoot is not noticed. The cause is quite evident as the osmotic effect of this solution even in the higher concentration (10 per cent.) used is not big enough to cause a sufficient shrinkage in the stem.

2. *Discoloration in the leaves*

The discoloration in the leaves is a clear sign of the death of the leaf-tissue. With all the three substances in solution, the discoloration is evident in the leaves. But the time taken for the discoloration to appear in the leaves, the places in the leaves at which it starts first, and the manner in which it spreads in the lamina are all different in the three cases. These differences depend on the mode of killing, characteristic of each substance and also on the capacity of the solute to diffuse out rapidly through the vessel walls.

With a solution of the non-permeable salt, sodium chloride, the discoloration starts at the tips of the leaves and in the margins near the tips. This discoloration resembles the one usually found in leaves of plants subjected to severe drought conditions. In both cases death of the cells appears to have been caused by desiccation. In higher concentrations of the solution, the discoloration appears sooner and in the lower concentrations, it is very much delayed or sometimes it is even absent.

With formalin solution, the discoloration is seen very early. It starts first along the main veins, slowly spreads along the minor veins and then to the other parts of the lamina. The characteristic appearance of the discoloration along the veins shows that the formalin diffuses out through the vessel walls quite rapidly. The discoloration appears much sooner with higher concentrations.

With the permeable salt, potassium nitrate, the discoloration is due to the accumulation of the salt in the cells beyond the critical concentration which is much higher than in the case of formalin. But with this salt solution, the discoloration starts in patches in the leaf lamina, especially near the tips of the ultimate veinlets. This appears to show that potassium nitrate does not pass out through the vessel walls. The cells along the main veins do not get discoloured even after three days in that solution. With this solution too, the discoloration appears much later than in formalin solution. With the higher concentrations of the potassium nitrate solution, the discoloration starts a little earlier.

These results show that the vessel walls are permeable to formalin but not to potassium nitrate. This differential permeability

of the vessel walls to different solutes in the sap rising in the vessels has, we believe, not hitherto been suspected.

Discussion

I. Osmotic effect of supplying a hypertonic solution to the cut end of a shoot on absorption and transpiration

When a hypertonic solution of a substance is supplied to the cut end of a shoot, its osmotic effect on the stem cells as well as on the leaf-cells brings about a fall in absorption. A fall in transpiration is also caused by the same effect in the leaf-cells. A fall in absorption or transpiration is due to a withdrawal of water from the living cells round the vessels by the osmotic solution in the latter. An extraction of water from the stem cells brings about a fall in absorption but not in transpiration except in very young and turgid shoots and with a supply of a high concentration of the solution to the cut end. In such cases, a large shrinkage of the stem caused by the withdrawal of water from the stem cells results in a mechanical constriction of the vessels bringing about a reduction in water-supply.

If the solute is non-permeable, the rate of absorption and transpiration tend to get steady and run parallel, after the solution has spread into the leaves. The concentration of the cell-sap in the leaf-cells is gradually increased due to the extraction of water from them by the osmotic solution in the vessels and also by evaporation of water from these cells to the outside. These factors enable the leaf-cells finally to absorb water from the vessels and allow it to be evaporated, thus establishing a fairly good equilibrium between absorption and transpiration. But still the two curves will be going down slowly as the solution in the vessels gets concentrated due to a continuous supply of the solution to the cut end.

Now, the conditions are altered, if the solute is permeable. In such cases, an increase in the osmotic value of the cell-sap of the leaf-cells is brought about in two ways: firstly by an extraction of water from them by the osmotic solution in the vessels and then by the diffusion of the solute into these cells. This increase in the osmotic value will enable these cells to absorb water from the vessels and make it available for evaporation. The rates of absorption as well as of transpiration are quite steady. Even a recovery in the two rates, from the previous low level brought about by the initial osmotic effect of the solution on the leaf-cells, would have taken place but for the gradual increase in concentration of the solution in the vessels in the leaves due to a continuous supply of the solution to the cut end, and also because an accumulation of the solute inside the cells beyond a certain limit proves generally toxic to the cells.

*II. Toxic effect of the solution on absorption
and transpiration*

The toxic effect of the solute brings about further changes in absorption and transpiration. When the solute gets accumulated in the cells beyond the critical concentration, the cells are killed. In the case of permeable salts like potassium nitrate, this critical concentration is quite high. Thus the killing of the leaf-cells with a supply of a solution of such a salt is a very slow process. But with a highly toxic substance like formalin, this critical concentration of the substance inside the cell necessary to cause its death, is low. Thus in such a case, before the osmotic effect of the solution on the leaf-cells can be complete, these cells are killed. With the death of the cells, they become permeable to some of the substances in the cell-sap. So these cells cannot keep up the same osmotic pressure as previous to their death so as to retain their cell-walls stretched to the same extent. Consequently there is a recoil of the cell-wall resulting in an expulsion of the cell-sap. The amount of sap released depends naturally on the turgor condition of the cell at the time of its death. The younger the cell and more turgid it is, the larger will be the amount of cell-sap released and *vice versa*. A release of the cell-sap from the leaf-cells during their death will expose the expelled sap directly for evaporation. This increases transpiration. But this statement requires further elucidation. Previous to the death of the leaf-cells, the rate of transpiration was at a low level because of the concentrated nature of the cell-sap, brought about initially by a withdrawal of water from these cells by the osmotic solution in the vessels. If such a concentrated cell-sap lowers transpiration when it is inside the cell, with the release of the sap, the transpiration rate must be kept at the same level or at least it cannot increase to such an extent as to bring it generally to the initial rate. But in the first case, where the cell-sap is inside the cell, the cell-wall itself controls the evaporation of the sap. In the latter case, where the sap is exposed direct to evaporation, this controlling factor, the cell-wall, is not present. But a more possible cause for the increased rate of transpiration, when the cell-sap is released to the outside, is that the concentration of this expressed sap is not the same as that of the original sap inside the cell. It is likely to be much less concentrated, *i.e.*, the dead cell may not be equally permeable to all the substances in the sap. Thus more of water and less of the solutes are probably sent out with the recoil of the cell-wall. Evaporation of this dilute sap exposed direct to the atmosphere will be higher than that of a concentrated sap inside the cell.

A second effect of the expulsion of the sap with the death of the cell is a loss in turgor and a lowering of the transpiration pull on the water in the vessels. With the increased rate of transpiration subsequent to the release of the sap, this pull is gradually

re-established and an increase in the rate of absorption is noticed, the recovered rate being more often about half the initial rate and sometimes even approaching the initial rate. This recovered rate keeps fairly constant for some hours. In the case of transpiration also, the rate is more or less equal to the initial rate and keeps fairly steady for some hours. These results show that with the death of the leaf-cells, the mechanism for transpiration and absorption is not destroyed. Such a recovery is possible only if the dead cells in the leaves can re-establish the transpiration pull by suction from above. Thus it has been assumed that the osmotic property of these cells is not completely destroyed nor is there a complete collapse of the cells, with their death. The dead cells are still able to absorb water from the vessels and make it available for evaporation.

This view is directly opposed to the present theory stated by Dixon (2), and supported by Overton (8) and many others. Dixon, when he supplied a poison to the cut end of a shoot, noticed a big fall in transpiration as well as in absorption when the poison was seen in the leaves. He says that this fall is due to the death of the leaf-cells. According to him, when these cells are killed by the poison, their osmotic property is completely destroyed and there is a collapse of the cells bringing about a reduction in the area of the transpiring surface and also a blocking of the intercellular spaces. All these factors contribute to the fall in transpiration and absorption. It is surprising to note that he did not notice an increased rate of transpiration with the death of the leaf-cells, which is a constant feature noticed in all the experiments described here, when the death of the cells is brought about by an accumulation of the substance in them. Nor does Dixon find a recovery in the rate of absorption as noticed in these experiments. Now Dixon's theory of collapse of the cells and complete destruction of their osmotic property cannot explain the fact that the rates of absorption and transpiration keep fairly near the initial rate for a long time after the death of these leaf-cells. This can be explained only by the assumption that the mechanism for absorption and transpiration in the dead cells is as nearly effective as in the living cells.

Further evidence is afforded in strengthening this assumption by the differences in the magnitude of the recovered rate and the period for which the recovered rate keeps constant. The magnitude will depend upon the amount of cell-sap inside the cells after their death or rather the amount of solutes in the cell-sap. The filtration of the sap during its expulsion to the outside with the death of the cell decides the concentration of the sap remaining in the cell. If the wall-pressure is not high at the time of the death of the cells, the amount of cell-sap released will be much less. But if the wall-pressure were high, the solutes retained in the cell will be less and the recovered rate will also be of a lower magnitude.

The further process of exosmosis of substances from inside the dead cells will gradually tend to lower the osmotic value of the sap of these cells. Thus the rates of absorption and transpiration go down slowly after sometime.

A fall and a rise in the rate of absorption, when a poisonous solution is supplied to the cut end, have not been noted till now. Ewart's results (6) on the effect of supplying formalin solution to the cut end of a tree on absorption, show a fall and a rise in the rate of absorption though not very clearly. But he did not explain this rise in the rate after the death of the leaves, though it amounted to about half the initial rate. This feature is common in all the experiments described in this paper, where formalin solution is supplied in different concentrations to the cut end of the shoot.

Another constant feature in the experiments shown here is an increased rate of transpiration during the death of the leaf-cells and a simultaneous fall in absorption. Overton (8) and Shroeder (10) found that the leaves lost 50 per cent. of their fresh weight very quickly when they were killed by supplying a poison to the cut end. They had not explained the causes for this phenomenon nor had they noted a simultaneous fall in absorption as they did not measure absorption. A simultaneous fall in absorption and an increased rate in transpiration during the death of the leaf-cells as recorded in these experiments make it possible to understand these features.

The effects of different concentrations of formalin supplied to shoots of different conditions bring out clearly a few points bearing on the influence of the condition of the shoot on absorption and transpiration. With the help of these data it could be more definitely established that the vitalistic theory of Sir J. C. Bose (1) cannot stand. He supplied 25 per cent. formalin to the cut end of a shoot of *Chrysanthemum* and noticed a continuous fall in absorption and a complete cessation of absorption after about 5 hours. He attributes it to the gradual killing of the "pumping cells" in the stem. It must be remembered that *Chrysanthemum* is a herbaceous plant. A fall in absorption as the solution is passing up the stem can be easily avoided by a selection of the shoot and of a proper concentration of the poisonous solution, as has been shown in the experiments described in this paper. It has been proved quite conclusively that a fall in absorption occurs only when the solution enters the leaves and kills the leaf-cells, provided proper precaution is taken in selecting a shoot, so as to avoid the initial fall. Even the fall in absorption with the death of the leaf-cells can be minimised by selecting a mature shoot, of low water-content and supplying a fairly low concentration of formalin to the cut end. Now the statement made by Bose that absorption comes to a standstill after about 5 hours is really surprising, for

such a result was not seen with any of the concentrations of formalin nor shoots of any particular description. But an extremely young and tender shoot of a herbaceous plant like *Chrysanthemum* may give this result mainly due to the complete collapse of the shoot and thorough and effective blocking of the vessels. On the other hand, if care be taken to avoid mechanical disturbance of the conducting paths, the rates of absorption and of transpiration after the death of the whole shoot tend to approach the initial rate and keep fairly constant for a long time.

III. Causes for the wilting of the leaves with the cut end of the shoot in formalin

A wilting of the leaves with the killing of the stem cells either by a poison or by some other method has been observed by a number of workers. Ursprung (13) steamed portions of the stem and found the leaves above drooping after sometime; the longer the stretch of the stem killed in that way, the sooner does the wilting take place. The same was observed by Strasburger but the latter did not attach much importance to it. But Ursprung based his vitalistic theory on these experiments. He maintained that the living cells in the stem take an active part in the ascent of sap. As, according to him, there was no blocking of vessels by internal deposits in all his experiments, he was sure that the wilting of leaves was due to a lack of water-supply brought about by the putting out of action of the living cells in the stem; especially as the wilting depended on the length of the stem killed. Boehm found a blocking of the vessels by internal deposits in his experiments with the same treatment. So did Dixon and Overton. These latter authors attribute the wilting to the blocking of the vessels with deposits. Ursprung (14) repeated his experiments but never found any blocking.

Sir J. C. Bose (1), when he supplied formalin (25 per cent.) to the cut end of a shoot of *Chrysanthemum*, first noticed a drooping of the leaves as well as a bending of the shoot tip before the poison could reach those parts. The whole shoot finally collapsed. He did not find any deposits blocking the vessels. The drooping of the leaves, according to him, is due to reduction in water-supply caused by the death of the "pumping cells" in the stem by the poison.

In the experiments described in this paper where different concentrations of formalin are supplied to the cut ends of the shoots, there is no blocking of the vessels; nor is there any wilting of the leaves. But when the formalin reaches the leaves, the leaves droop down. This is very clear in young and turgid shoots, especially in the higher concentrations. This drooping of the leaves is similar to that noticed with a higher concentration of sodium chloride solution or potassium nitrate solution.

With a young and turgid shoot, when a high concentration of formalin is supplied to the cut end, the shoot collapses at the top nodes partly due to the osmotic extraction of water and partly to the death of the cells in the nodes. This occurs even with a supply of a higher concentration of sodium chloride solution to the cut end of a shoot, but here it is a purely osmotic effect. With old shoots, this collapse of the shoot does not occur in either of the solutions.

These two effects, *i.e.*, collapse at the nodes and drooping of the leaves are quite distinct from the wilting of the leaves noticed by the other workers. Only in very young and turgid shoots, with a supply of a high concentration of formalin to the cut end, do the wilting of the leaves and the bending of the shoot-tip take place. This occurs even if a high concentration of sodium chloride solution is supplied to the cut end of such a shoot. The wilting occurs before the solution could reach those regions. This wilting is certainly due to the large shrinkage in the stem caused by the osmotic withdrawal of water from the stem tissue as the highly concentrated solution is passing up the stem. This big shrinkage causes a mechanical compression of the vessels, thus not allowing a normal conduction of the sap in them. This naturally results in a wilting of the shoot tip. This fact was recognised by Ursprung (14) also. He says "Die Funktion der lebenden Zellen kann eine verschiedene sein, sie haben entweder die Aufgabe die leitenden Elemente in leitungsfähigen Zustand zu erhalten oder aber einen Teil der Hebungskraft zu liefern. Die Hauptfunktion ist für höhere pflanzen die letztere, während vielleicht bei niedern Kräutern die Erhaltung des leitungsfähigen Zustandes der Leitungsbahnen zur Hauptfunktion werden kann." But in the previous paper it has been proved by quantitative experiments that the stem cells do not take any part in the ascent of sap and now it is seen that the living cells round the vessels are necessary to keep the latter in a distended condition to allow a normal flow of sap in them.

The wilting of the shoot tip as well as a drooping of the leaves noticed by Sir J. C. Bose in his experiments on *Chrysanthemum* are due no doubt to a reduction in water-supply, though it is attributed by him to the death of the "pumping cells" in the stem. But really the reduction in water-supply is brought about by a mechanical constriction of the vessels due to a shrinkage of the stem tissue. It must be remembered that *Chrysanthemum* is a herbaceous plant with little mechanical tissue so that any shrinkage will affect water-condition badly.

Ewart, after killing the stem of a tall tree by supplying the cut end with formalin solution found that coloured solution, which was supplied later to the cut end, rose up the stem through the older vessels and not through the latest formed vessels, which were the original paths for normal conduction. In the living condition, the

latest formed young vessels offer least resistance for normal conduction. After the death of the surrounding cells, the same vessels offer greater resistance to the flow than the older vessels and so the liquid now rises up in these latter vessels though at a much slower rate. It is thus evident that even in the trees, the youngest vessels are maintained in a proper condition by the living cells round them and the death of these cells increases the resistance of vessels for normal conduction of sap in them.

IV. Discoloration of the leaves

There is some literature as to the discoloration of the leaves with the death of the leaf-cells. Ursprung finds discoloration of leaves in patches in a few cases when a part of the stem is steamed and thus killed. Roshardt (9) observes discoloration in the leaves as a result of steaming or poisoning the stems. Dixon finds a similar discoloration in patches in leaves when a part of the stem has been steamed. Overton also gets the same discoloration with the same treatment. These two latter authors are of the opinion that the discoloration is due to a poisonous or plasmolysing substance which has been exuded into the vessels from the steamed portion of the stem and when it reaches the leaves, it kills the leaf-cells thus causing the discoloration.

In the experiments described in this paper, there are three kinds of discoloration of the leaves. With a supply of sodium chloride solution to the cut end, after a long time, discoloration of the leaves starts at the tips and the margins near the tips and slowly spreads inward. This is the kind of discoloration seen in leaves of plants which are subjected to drought conditions for a long time. The discoloration in both the cases appears to be due to a lack of water-supply.

With a supply of formalin solution to the cut end, when it enters the leaves, the discoloration starts along the main veins first, then to the lateral veins, and finally spreads to other parts of the lamina. With potassium nitrate solution, the discoloration starts much later in patches between the veins in the leaves and spreads to other parts of the lamina, last of all to the veins. In both these cases the discoloration is caused by the killing of the leaf-cells due to an accumulation of the substance in the cells. The nature of the discoloration in both the cases appears to show that formalin can pass readily through the vessel walls while potassium nitrate does not pass through them. This means that the vessel walls are permeable to formalin and non-permeable to potassium nitrate. If it is so, then the question arises whether there exists a differential permeability of the vessel walls for the solutes in the sap passing in the vessels. It appears this line of investigation will give interesting results as to the passage of salts laterally in the stem, also in the leaves.

Finally mention must be made of the influence of the water-content and age of the shoot. From the results obtained, it can be easily seen to what a great extent this factor controls the effect of the poison or salt solutions on absorption and transpiration.

Summary

1. Further observations on the effects of three different substances, *viz.*, formalin (a poison), sodium chloride (a non-permeable salt), and potassium nitrate (a permeable salt), in different concentrations, on absorption and transpiration when applied to the cut end of a shoot of *Barleria cristata*, are recorded.

2. The effects are noted during three stages in the passage of the solution up the shoot, *viz.*, (i) when the solution is passing up the stem, (ii) as it enters the leaves and (iii) after its entry into the leaves.

3. As regards absorption, all the three substances bring about a fall in the rate in the first stage. In the second stage, the fall is accelerated, the magnitude depending on the concentration and the age and water-content of the shoot. Though the apparent result is the same in all cases at this stage, the factors operating in each case are shown to be different. In the third stage, different effects are noted. With sodium chloride, the fall continues; with potassium nitrate, the fall is very much reduced; and with formalin there is a rise which may equal the initial rate. The causes for these differences are discussed.

4. As regards transpiration, the rate is not affected during the first stage. During the second stage, at first a fall is noticed in all cases, its magnitude depending on concentration and age and water-content of the shoot. But differences appear very soon. With sodium chloride, the fall continues; with potassium nitrate, the fall is arrested and the rate becomes steady at a lower level, and with formalin a rise in the rate occurs. During the third stage, with sodium chloride, the fall gets slower, with potassium nitrate, a slow rise occurs and with formalin, the recovered rate is kept up. The results are explained and the mechanism is discussed.

5. The relation between absorption and transpiration with different treatments is discussed.

6. Observations of previous workers are discussed in the light of the results obtained in the present experiments.

7. Bose's observations on *Chrysanthemum* are shown to be not due to the death of the 'pumping cells'.

8. Further evidence is adduced in support of the assumption that the osmotic property of the leaf-cells is not destroyed by death.

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THE OSMOTIC PRESSURE AND THE H-ION
CONCENTRATION
OF
SEAWEEDS IN RELATION TO THOSE
OF SEA WATER

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Received for publication on 7th July, 1934.

Very little is known of the conditions of algal growth and the factors governing their distribution in space and time and practically no knowledge exists about the relation of algae to the physical environment. The knowledge bearing on these aspects of algal population is both academically and economically important. At the end of every nutritive chain there are plants of one kind or the other which manufacture food from inorganic materials with the energy of sunlight. The fishes ultimately depend for their food on the seaweeds and if we value fish we should be interested in the success of their food also. Secondly seaweeds are directly used for the preparation of gelatines, agar agar, iodine and potash and as a kind of fertilizer.

Nothing is known about the algal flora of the Bombay Island. Attempts have recently been made to study the algal flora from a systematic point of view and scanty information is available about their general distribution except in the case of some common species of seaweeds on account of the labours of Iyengar (1933a, 1933b, 1933c), Dikshit S. C. (1931) and Boergesen (1930-1932).

The work of identifying the species of the algae is difficult and it would take a very long time before the marine algal flora of the Bombay Island is thoroughly worked out. It is necessary that the relation of alga to their physical environment should be studied without waiting for the knowledge of composition of the algal flora. These determinations are of importance as the vertical and horizontal distribution of algae depend on the conditions of the habitat. No work was done before and it is considered important to make a beginning in that direction. The town of Bombay lies at latitude 75° 75' E., longitude 12° 20' N. The town is surrounded by the waters of the Arabian sea.

The beaches near Colaba and Kennedy sea face and Juhu are flat sandy stretches while the beaches near Bandra creek, Thana and a portion near Colaba facing the Reclamation are muddy and rocky.

The sandy and rocky beaches near Colaba are very rich in algal flora while the beach near Chowpatty and Juhu are rather destitute of algal species. Algae are generally found on beach at a distance of a mile and a half towards the sea. Some are found on the beach exposed to air during the ebb while some are found in pools with sandy or muddy bottoms between the rocks. It is not possible to mention the number of species found and to determine the percentage of each class of algae in the total number of species growing here. It is also not possible to say anything about the relative richness of the Bombay algal flora as compared to other places in the Bombay Presidency. The most striking characteristic of the Bombay algal flora appears to be the preponderance of the red algae.

The principal factors affecting the growth and distribution of algae are generally the temperature, light, composition of sea water, turbidity, movement of water and the nature of the habitat. Setchell (1915) has shown that the majority of species of algae occur in regions having a range of not more than 10°C and that those occurring in regions having a greater range than this accommodate themselves to the general law by their seasonal distribution. He has distinguished different regions based on the average temperature of water during the summer. The effect of light and turbidity of water on the vertical distribution of marine algae at Beaufort is studied by Hoyt (1917-1918) and he finds that the vertical distribution is exceedingly limited, the total range for all the species being only about 2.2 metres from the usual high-tide time as the water contains a considerable amount of suspended matter which consequently reduces the light intensity. The study of the chemical and physical factors of sea water that govern the distribution of algae is made by Gail (1918, 1919). He has shown the effect of light and H-ion concentration on the growth of *Fucus evanescens* Ag. and he has pointed out how the presence of *Ulva lactuca* L. raises the pH value of water and thus prevents the growth of *Fucus evanescens* Ag. even though other conditions of life are normal. There is a large amount of work done on the pH value of water. It is an important factor that governs the various life processes of animal and plant organisms and ultimately their distribution. The pH value of sea water depends on its oxygen content as is shown by Helland-Hansen (1914). The Photosynthetic activity of algal flora in summer raises the oxygen content of the sea water and consequently the pH value of sea water is on the alkaline side during summer while it is lower during the winter months. The high alkalinity of sea water is thus produced by the photosynthetic activity of Plankton and attached algae. Similar results are obtained by McClendon (1917), showing a lower pH value in the morning and a higher pH value in the evening sea water. H-ion concentration of sea water is also affected by temperature. Mayer (1919) and others have found that for every fall in temperature by one degree there is a fall in the pH of water by 0.01.

The effect of various constituents of sea water like calcium and magnesium salts on the pH value of sea water is also studied. The presence of magnesium-hydroxide in sea water raises its pH value to higher degree than calcium hydroxide though the OH-ions concentration producible by the former salt is less than that of the latter. This is very probably due to the lesser solubility of calcium carbonate than that of the magnesium salt. The study of the effect of a salt, when present in a mixture, on the H-ion concentration is extremely difficult on account of various interactions between the salts present.

Atkins (1922, 1922a, 1923, 1924) has made an exhaustive study of the H-ion concentration of sea water at Plymouth and has found that the sea water may become as alkaline as pH 9.7 as a result of very active photosynthesis. The pH value of sea water of the Sound changes between the high

tide and the low tide. Similarly the sea water surrounding the algae is more alkaline than that of the general mass of water. The H-ion concentration of sea water shows variations during the year. Atkins (1922a) has studied the H-ion concentration of the cells of the marine algae. He has used either the sections of the thallus or the expressed sap from the cells. His results show that the reactions of the sap in various groups of algae is in most cases neutral. He has also shown that increased alkalinity of sea water is injurious to *Ceramium rubrum* Huds. very probably due to the increased permeability of superficial cells.

The above review indicates that certain amount of work is done on the effect of external factors on the growth and distribution of the marine algae and from the results it appears that H-ion concentration of sea water is one of the most important factors governing the growth and distribution of seaweeds. No such determination of the H-ion concentration of the sea water has been made in the tropical seas and we therefore undertook to determine the pH value of sea water near the shores of Bombay and to determine the pH value of the cell sap of the common seaweeds.

A large amount of information is available on the osmotic concentration of the cell sap of various plants in relation to the physical environment but, so far as the writers are aware, no information exists about the osmotic concentration of the cell sap of marine algae. The marine algae are surrounded by waters of high degree of salinity and therefore they are subjected to a different type of aquatic environment than the fresh water forms. The determinations of the concentration of the sea water were not done before, and so it would also be of interest to study the osmotic pressure of sea water at different times of the year and to investigate their relations with the osmotic pressure of the seaweeds.

Determination of the H-ion Concentration of the Cell-Sap of the Seaweeds and of the Sea Water

The H-ion concentration of the cell sap of the seaweeds and the sea water was determined by the calorimetric method given by Clark (1920). Lubs and Clark (1916) have improved upon the method of the preparation of Sulphonthalein indicators and the stock solutions of the eight indicators were prepared accordingly.

Method of Extraction of Cell-Sap

The algal thalli are cut into small pieces by means of a highly polished pair of steel scissors and the pieces are put in test tubes. The test tubes are plugged with paraffined cork stoppers and plunged into salt ice mixture and frozen as quickly as possible in order to prevent any appreciable chemical change occurring there either by enzyme action or by oxidation. The test tubes are allowed to remain in the freezing mixture for half an hour. The test tubes are placed in water for about 5 to 10 minutes to allow the material to thaw. The material is then removed from the test tubes and the cell sap extracted by means of a silver-plated hand crusher. The cell sap is directly collected in Jena glass test tubes.

For the determination of the pH value 10 cc. of the extracted sap are taken in a Jena glass tube of 16 mm. internal diameter, and 0.5 ccs. of the indicator is added as required. In case of thymol

blue and methyl red 1 cc. and 0.3 cc. respectively of the solution are taken. The colour of the cell sap is then matched either with the colours in the colour chart of Clark and Lubs indicators in solutions of known pH or with the standard solutions of borax-boric acid mixtures prepared. The pH value obtained by one indicator is again tested by another indicator whose pH range includes the value obtained by the indicator used first.

The H-ion concentration of the sea water is determined in the same manner described above. The sea water is not filtered before using, for filtering would alter the pH value by the absorption of salts by the filter paper. The pH value of the sea water is determined immediately after collection so as to prevent alteration of pH value after collection. In some cases the sea water is collected from pools and in muddy places so as to study the differences in its pH value from the general mass of water. The determination of the pH value of the sea water are made during the months of February and May. The following Table I gives the pH value of the sea water during these months:—

TABLE I
pH value of the sea water near Bombay

Date.	Time.	pH value.	Nature of the place of collection.	Remarks.
8—2—1932	12-30 noon	8.31
13—2—1932	9-00 a.m.	8.31
14—2—1932	10-00 a.m.	8.31
15—2—1932	1-00 p.m.	8.41
18—2—1932	9-45 a.m.	8.31
20—2—1932	9-00 a.m.	8.21	Muddy places.	..
23—2—1932	2-00 p.m.	8.31
24—2—1932	..	8.31
25—2—1932	..	8.31
2—3—1932	11-45 a.m.	8.31
4—3—1932	11-00 a.m.	8.31
6—3—1932	4-00 p.m.	8.20
9—3—1932	3-45 p.m.	8.31

TABLE I—*contd.*

Date.	Time.	pH. value.	Nature of the place of collection.	Remarks.
10-3-1932	12-00 noon	8.31
13-3-1932	4-00 p.m.	8.31
16-3-1932	6-30 p.m.	8.31
17-3-1932	9-45 a.m.	8.20	Muddy places.	..
18-3-1932	10-30 a.m.	8.31
20-3-1932	12-00 noon	8.31
25-3-1932	9-45 a.m.	8.31
28-3-1932	11-45 a.m.	8.31
29-3-1932	12-30 a.m.	8.31
30-3-1932	1-30 p.m.	8.31
7-4-1933	9-15 a.m.	8.31	..	High tide.
8-4-1933	9-00 a.m.	8.31
9-4-1933	9-30 a.m.	8.31
19-4-1933	8-00 a.m.	8.31
4-5-1933	2-00 p.m.	8.31
5-5-1933	3-00 p.m.	8.31
6-5-1933	1-00 p.m.	8.31
8-5-1933	2-00 p.m.	8.20
10-5-1933	1-30 p.m.	8.20

The measurements of the pH value of sea water is generally fluctuating between 8.21 to 8.31 (Table I). Out of 32 determinations of the pH values made on different days and from different localities twenty-six determinations gave the pH value of 8.31 indicating that the pH value of sea water near Bombay is 8.31. The other pH values are 8.21 and 8.41. The sea water is distinctly alkaline and the value obtained very nearly agreed with the pH value of sea water obtained by other workers abroad. It was not possible to make hourly determinations for twenty-four hours for want of

facilities but the sea water collected at different times of the day did not show differences.

Table II gives the pH value of the cell sap of two species of Chlorophyceae and two species of Phaeophyceae. The determinations were made from January to March when these species were found in large quantities on the beach. The pH value of *Enteromorpha* sp. fluctuates between 6.6 and 6.8. It appears from the results that the pH value of the seaweed does not vary. The cell sap is nearly neutral, while the sea water is alkaline. The pH value of *Ulva lactuca* L. also fluctuates between 6.6 and 6.8. In *Sargassum bacciferum* L. the pH value fluctuates between 6.4 and 6.6. It shows no difference from the species of Chlorophyceae. In the other species belonging to Dictyotaceae the cell sap is remarkably acidic. The pH value is as low as 2.0 and fluctuates between 2.0 and 2.8.

TABLE II
pH value of the cell-sap of two species of
Chlorophyceae and two species of Phaeophyceae

Date.	pH value of <i>Enteromorpha</i> sp.	pH value of <i>Ulva lactuca</i> L.	pH value of <i>Sargassum</i> <i>bacciferum</i> L.	pH value of <i>Dictyota</i> <i>Atomaria</i> Flanck.
13-1-1933	6.6	6.6
17-1-1933	6.4	..	6.4	..
18-1-1933	6.6	..
13-2-1933	6.8	6.8
14-2-1933	6.8
15-2-1933	6.8	6.8
18-2-1933	6.8	2.4
22-2-1933	6.6	7.0	..	2.4
23-2-1933	6.6	6.8	..	2.4
24-2-1933	6.8
27-2-1933	6.8	6.6	6.6	2.8
2-3-1933	6.8	..	6.6	..
3-3-1933	6.8	7.0
4-3-1933	6.8	6.6	..	2.0

TABLE II—*contd.*

Date.	pH value of <i>Enteromorpha</i> sp.	pH value of <i>Ulva lactuca</i> L.	pH value of <i>Sargassum</i> <i>bacciferum</i> L.	pH value of <i>Dictyota</i> <i>Atomaria</i> Flanck.
6—3—1933	6·8	2·8
7—3—1933	6·8	6·6	..	2·8
8—3—1933	6·8	6·8	..	2·4
10—3—1933	6·6	6·8	6·6	2·8
13—3—1933	6·6	6·8	6·6	..
15—3—1933	6·6	6·8	6·4	2·2
16—3—1933	6·6	6·8	6·4	2·0
17—3—1933	6·8	6·8	6·4	2·8
18—3—1933	6·6	6·6	6·6	2·8
20—3—1933	6·6	7·0	6·8	2·8
22—3—1933	6·6	6·8	..	2·6
23—3—1933	6·8	6·6	..	2·8
25—3—1933	6·6	6·6	6·4	2·8
27—3—1933	2·8
29—3—1933	6·6	6·6	6·4	2·8

TABLE III
pH value of the cell-sap of some species of
Rhodophyceae

Date.	pH value of <i>Galaxaura</i> sp.	pH value of <i>Polysiphonia</i> sp.	pH value of <i>Rhodomenia</i> <i>laciniata</i> Huds.	pH value of <i>Acanthophora</i> sp.
13—12—1932	7·0
13— 1—1933	6·6
17— 1—1933	6·2
18— 1—1933	..	6·6	..	6·4
13— 2—1933	..	6·8	..	6·6

TABLE III—*contd.*

Date.	pH value of <i>Galaxaura</i> sp.	pH value of <i>Polysiphonia</i> sp.	pH value of <i>Rhodymenia</i> <i>laciniata</i> Huds.	pH value <i>Acanthophora</i> sp.
14-2-1933	..	6.4	..	6.6
15-2-1933	..	6.4	5.2	..
18-2-1933	..	6.4	6.4	..
21-2-1933	..	6.6	..	6.6
22-2-1933	..	6.4	..	6.6
23-2-1933	6.2	6.6	6.8	6.6
24-2-1933	..	6.4	..	6.6
27-2-1933	6.4	6.4	6.4	6.6
2-3-1933	..	6.4
3-3-1933	6.6	6.4
4-3-1933	6.8	..	5.2	..
6-3-1933	6.2	..
8-3-1933	6.4	..
10-3-1933	6.4	6.4
13-3-1933	6.6	..	6.6	..
15-3-1933	6.6	6.6	6.4	..
16-3-1933	6.6	..	6.4	..
17-3-1933	6.6	..	6.6	..
18-3-1933	6.6	..	6.6	..
22-3-1933	6.6	6.6
23-3-1933	6.6	6.6
25-3-1933	6.0	..	6.4	6.2
27-3-1933	6.4	6.2
28-3-1933	6.6	..	6.4	..
29-3-1933	6.4

In the Table III the pH values of the cell sap of four species of Rhodophyceæ are given. The pH values of all the four species

are remarkably uniform. The range of pH value is 6.2 to 6.8. In two cases in *Rhodomenia laciniata* Huds. the pH is as low as 5.2, otherwise in the rest of the determinations the pH value fluctuates between 6.2 and 6.8.

It is a significant fact that the cell sap of algae is more or less neutral while the cell sap of higher plants is generally acidic in nature. The lesser acidity of the cell sap may be due to the alkalinity of the sea water in which they remained immersed.

Determination of Osmotic Pressure of the Sea Water

There are different Physical methods for determining the osmotic pressures of salt solutions. The osmotic pressure of the solution can be determined either by the vapour pressure method, the freezing point method, the boiling point method, direct method or critical solution temperature method. The sea water contains a heterogeneous mixture of salts in different proportions, and so it is necessary to determine the osmotic pressure by some other method, as these physical methods cannot be used on account of the difficulty of finding out the molecular weights of the salts in sea water. It is therefore undertaken to determine the osmotic pressure of sea water by the plasmolytic method. The principle of the method is to determine the osmotic pressure of some vegetable cells by means of sucrose solutions of known normality and then determine the concentration of the original sea water that would balance the osmotic pressure of the cell sap of the same kind of vegetable cells. In order to balance the osmotic pressure of the cell sap of the vegetable cells it is necessary to dilute or concentrate the sea water and the osmotic pressure of the sea water is calculated accordingly. This is not a very accurate method as it is a known fact that osmotic pressure of even a pure solution of a salt is not strictly proportional to its dilution. But the error involved in such calculations will not be significant for the purpose of the investigation. Secondly, the results obtained by this method are again verified by the freezing point method and it is noticed that there is good agreement in the results obtained by the two methods. Epidermal cells of the leaves of *Tradescantia discolor* L'Hér. are used. The differences in the osmotic pressure of the cell sap of the cells situated at different places on the leaf and in different leaves are noted and after a certain amount of trial the epidermal cells lying in the middle region of the mature leaf are always selected for use in these determinations. The osmotic pressure of the cells is first determined by the plasmolytic method using sucrose solutions. The cells from the same region of the leaf are taken and the osmotic pressure of the cell sap is determined by plasmolysis with diluted or concentrated sea water and the osmotic pressure of the sea water

is then calculated from the osmotic pressure of the sucrose solutions used in the previous experiments. The sucrose solutions used ranged from 0.2N to 0.3N with an interval of 0.01N. The sea water had to be diluted to about five times its original volume.

As the osmotic pressure of the sea water fluctuated within very narrow limits during the year the determinations are not beset with difficulties and the error arising from the differences in the osmotic pressures of the cells used is greatly minimised on account of the lesser number of repetitions involved in the measurements.

The osmotic pressure of the sea water is also determined by the freezing point method. As the sea water contains a mixture of salts the ordinary freezing point method cannot be used, as the molecular weights of the salts in the sea water are not known and as the degree of ionisation of different salts is different. For this reason the ordinary formula for determining the osmotic pressure cannot be used for measuring the osmotic pressure of the cell sap of plants, as the molecular weights of the substances in the cell sap are not known and therefore the osmotic pressure by the depression of the freezing point method cannot be determined. In order to overcome this difficulty various workers have determined the relations between the osmotic pressure in atmospheres and the depression of the freezing point in degrees centigrade of a solution of a normal solute in water and 12.05 which is the value of the ratio of the two quantities is generally taken. Perhaps this method of determining the osmotic pressure of a solution containing a heterogeneous mixture of salts of unknown molecular weights is more inaccurate than the plasmolytic method employed here, as value of the ratio will be far from the actual value on account of the complicating factors involved. But, inaccurate as this method is, it would be useful in checking the results obtained by the above method not employed previously in such determinations and it would be interesting to see how the results obtained by the plasmolytic method differ from those obtained with the freezing point method.

Firstly the freezing point of the distilled water is determined by using a Beckman's thermometer employing usual procedure. The level of the mercury first falls, rises up again and then remains constant for a short time, and then falls again. The point where the mercury remains constant for the short time gives the freezing point of water. Distilled water is then replaced by the sea water and the freezing point is determined. The osmotic pressure of the sea water is then calculated by using the formula $(T - T_1) \times 12.05 = \text{osmotic pressure}$, where T is the freezing point of distilled water, T_1 the freezing point of the sea water and 12.05 is the ratio of the osmotic pressure expressed in atmospheres to the freezing point depression in degrees centigrade of a solution of normal solute in water.

The osmotic pressure of the sea water immediately after collection is measured by the plasmolytic method as well or by the freezing point method from the months of October to May. This is the period when the sea weeds are generally found on the beaches near Bombay.

The following Table IV gives the value of the osmotic pressure of sea water on different days determined by the indirect plasmolytic method:—

TABLE IV

Date of determination.	O. P. of sea water in atms.
7—10—1932	24·80
21—10—1932	24·80
2—11—1932	24·60
21—11—1932	24·60
30—11—1932	24·60
7—12—1932	24·80
14—12—1932	25·00
15—12—1932	24·60
13— 1—1933	24·60
8— 2—1933	25·00
13— 2—1933	24·80
20— 2—1933	24·80
21— 2—1933	24·80
23— 2—1933	24·80
25— 2—1933	25·00

The results show that the osmotic pressure of sea water lies between 24·6 to 24·80 atmospheres. The osmotic pressure of sea water is also determined by the freezing point method side by side. The results are given in the Table V on the next page.

The osmotic pressure of the sea water as determined by the freezing point method is higher than the osmotic pressure deter-

mined by the plasmolytic method. The osmotic pressure of sea water varies from 25 atms. to 26 atms. These results cannot be considered very accurate as the sea water contains a mixture of salts but these results do give some idea of the osmotic pressure of sea water.

TABLE V
Osmotic pressure of Sea Water by the freezing point method and the indirect plasmolytic method

Date of determination	O.P. of the sea water by the freezing point method in atms.	O.P. of the sea water by the plasmolytic method in atms.
1—3—1933	25.4880	..
2—3—1933	25.1845	..
3—3—1933	25.9075	..
4—3—1933	24.9435	24.80
5—3—1933	24.8230	..
9—3—1933	26.0260	25.00
10—3—1933	24.9435	24.66
11—3—1933	25.6665	25.00
14—3—1933	..	24.60
15—3—1933	25.425	24.80
21—3—1933	..	25.00
23—3—1933	25.546	..
25—3—1933	26.0280	..

Determination of the Osmotic Pressure of the Marine Algae

Ordinary plasmolytic method is employed for determining the osmotic pressure of the seaweeds. The species belonging to three main classes of algae are selected. The osmotic pressure of the cells of each species is determined soon after collection. The weeds are brought in to the laboratory in the sea water and the osmotic pressure of the sea water as well as of the algae determined. The

Tables VI to VIII give the osmotic pressure of the species of Chlorophyceae, Phaeophyceae and Rhodophyceae, respectively:—

TABLE VI
Osmotic pressures of the species of Chlorophyceae, Phaeophyceae and Rhodophyceae in atmospheres

Date	Osmotic pressures of the cells of:—					
	<i>Ulva lactuca</i> L.	<i>Enteromorpha</i> sp.	<i>Padina tetrastrumata</i> Hauck.	<i>Sargassum bacciferum</i> L.	<i>Galaxaura</i> sp.	<i>Acanthophora</i> sp.
12—1—1932	..	33·0
13—1—1932	39·2
14—1—1932	36·0	33·0
15—1—1932	39·2	33·0
16—1—1932	36·1	39·1
17—1—1932	39·1	39·1	..
20—1—1932	40·0	36·2	..	39·1	41·0	..
21—1—1932	39·1	36·2	30·0
22—1—1932	..	39·1
23—1—1932	33·1	..	39·1	..
25—1—1932	36·1
26—1—1932	36·2	30·1	..	36·1
27—1—1932	36·2	33·1
28—1—1932	39·9	27·4	..	41·0
29—1—1932	..	39·1
30—1—1932	..	39·1
1—2—1932	33·2	39·1	39·1
2—2—1932	36·1	..	33·2
3—2—1932	36·1	36·1	33·2
4—2—1932	..	39·1	39·1
9—2—1932	32·2	..	30·1
10—2—1932	36·1	..	36·1	..	36·2	..

TABLE VI—*contd.*

Date.	Osmotic pressures of the cells of:—					
	<i>Ulva lactuca</i> L.	<i>Enteromorpha</i> sp.	<i>Padina tetrastrum</i> Hauck.	<i>Sargassum bacciferum</i> L.	<i>Galaxaura</i> sp.	<i>Acanthophora</i> sp.
11-2-1932	36.2	..	36.2	41.0	30.2	..
12-2-1932	33.2	42.0
13-2-1932	33.2
15-2-1932	36.2	36.2	36.2
16-2-1932	33.2
17-2-1932	36.2	..	39.2	42.0
18-2-1932	39.2	..	42.0
19-2-1932	39.2	..	39.2
22-2-1932	..	39.2
23-2-1932	..	39.2	..	42.0
24-2-1932	42.0	39.2	36.2
25-2-1932	39.2	36.2	36.2
26-2-1932	39.2	42.0	42.0
27-2-1932	39.2	42.0	42.0
1-3-1932	42.0	42.0	39.2
2-3-1932	36.2	36.2	36.2	..	36.2	..
3-3-1932	36.2	36.2	45.0	..	39.2	..
4-3-1932	48.2	36.2	48.0
5-3-1932	41.2	..	45.0
7-3-1932	39.2	39.2	39.0
8-3-1932	..	36.2
23-3-1932	48.0	48.0
24-3-1932	48.0	48.0
28-3-1932	42.0	42.0
29-3-1932	45.0	48.0

TABLE VI—*contd.*

Date.	Osmotic pressures of the cells of:—					
	<i>Ulva lactuca</i> L.	<i>Enteromorpha</i> sp.	<i>Padina tetrastronotica</i> Hauck.	<i>Sargassum bacciferum</i> L.	<i>Galaxaura</i> sp.	<i>Acanthophora</i> sp.
31—3—1932	..	45·0
1—4—1932	45·0	45·0
2—4—1932	45·0	45·0
3—4—1932	39·2	48·0
4—4—1932	45·0	42·0
7—4—1932	39·0	42·0
8—4—1932	42·0	42·0	45·0	..
25—4—1932	45·0	45·0	45·0	..
26—4—1932	48·0	48·0	45·0	..
27—4—1932	45·0	45·0	45·0	..
28—4—1932	45·0
29—4—1932	48·0	45·0	42·0	48·0
30—4—1932	48·0
1—5—1932	45·0
3—5—1932	48·0	48·0	48·0
4—5—1932	45·0	45·0	45·0
7—5—1932	45·0	..	45·0	45·0
8—5—1932	42·0	..	48·0	48·0
9—5—1932	42·0	..	45·0	45·0
11—5—1932	48·0	..	48·0	45·0
12—5—1932	48·0	..	48·0	48·0

Two important conclusions can be drawn from the study of the results given in the above Table VI.

(1) The osmotic pressure of the seaweeds is always higher than the osmotic pressure of the sea water, and

(2) The osmotic pressure in the seaweeds rises from October to May. There is no progressive rise indicated in the results as the values for the osmotic pressure are higher in the beginning at some places but these higher values may be due to many causes. It is

difficult to determine the age of the plant. If the algae are exposed to air for larger periods before collection there will be a change in the osmotic pressure. The osmotic pressure of the different cells of a plant may also vary. These irregularities disappear if the averages of each week's determinations of the osmotic pressures of each species are taken.

The osmotic pressure in the *Ulva lactuca* L. rises from 38.7 atms. to 46.0 atms. from January to May. Similar is the case with *Enteromorpha* sp. where it goes up from 33.0 atms. to 45.7 atms. Similar rise in the osmotic pressure is visible in each remaining species of algae investigated.

The graphs showing the osmotic pressure of the different species in different months of the year are given in Figure 1.

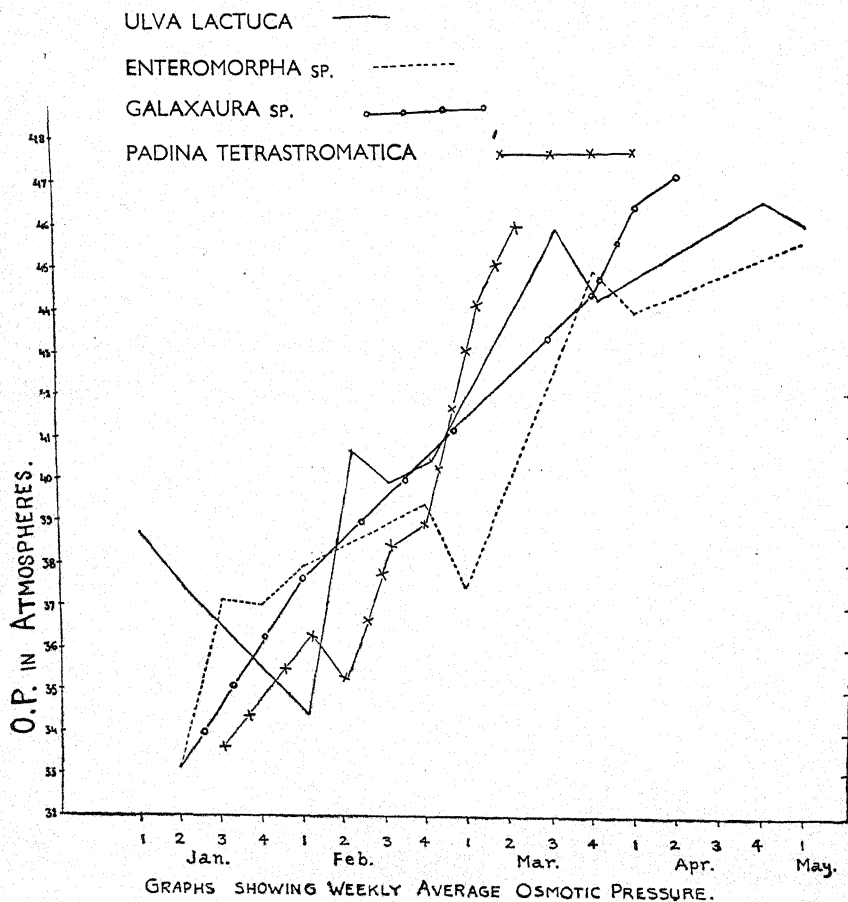


Fig. I

Summary

Hydrogen-ion concentration of the sea water is one of the most important factors that determine the distribution of the algal forms and a study of the relations between H-ion concentration of the sea water and of the algal forms was undertaken. The relation between the osmotic pressure of the algal cells and of the sea water must also be important and as no attempt was made before to study them, a study of this relation also was taken up.

The H-ion concentration of the sea water is studied by the indicator method as developed by Clark (1920). The same method is adopted for the determination of H-ion concentration of the cell sap of the algae. A method is developed for determining the osmotic pressure of the sea water and the results obtained verified periodically by the physical method of the lowering of the freezing point. The osmotic pressure of the algal forms is determined by the plasmolytic method.

The species of algae investigated are the *Ulva lactuca* L., *Padina tetrastromatica* Hauck., *Saragassum bacciferum* L., *Rhodomenia laciniata* Huds., *Enteromorpha* sp., *Acanthophora* sp., *Polysiphonia* sp., and *Galaxaura* sp.

The pH value of the sea water is about 8.31 and that of the sea water in pools varies from 8.0 to 8.21. The pH value of the cell sap of all the algal species examined is remarkably uniform. It fluctuates between 6.6 to 7.0. These results indicate that the cell sap of the algae is nearly neutral. This is a point of importance as generally the cell sap of higher plants is acidic in nature.

The osmotic pressure of the sea water is determined by the plasmolytic method. The range of fluctuation is very great. In pools it is higher, about 26 atms. and in very muddy places it may be as high as 33 atms. The osmotic pressure of the sea water determined by the freezing point method varies from 24.8 to 26 atms.

The osmotic pressure of the algal forms is always higher than that of the sea water. The range of fluctuation is very great. In algal forms in muddy water it is generally very high. The osmotic pressure of the forms lying out of water on the beach is also very high. In sea water, the osmotic pressure of *Ulva lactuca* L. is nearly 36.2 atms., in muddy water, it is 39 atms. and in forms exposed to air, it is 41 atms. The same holds good for other species.

The authors record here their deep sense of gratitude to Professor R. H. Dastur, Head of the Botany Department, The Royal Institute of Science, Bombay, for his able guidance and for offering us all the facilities that he could command.

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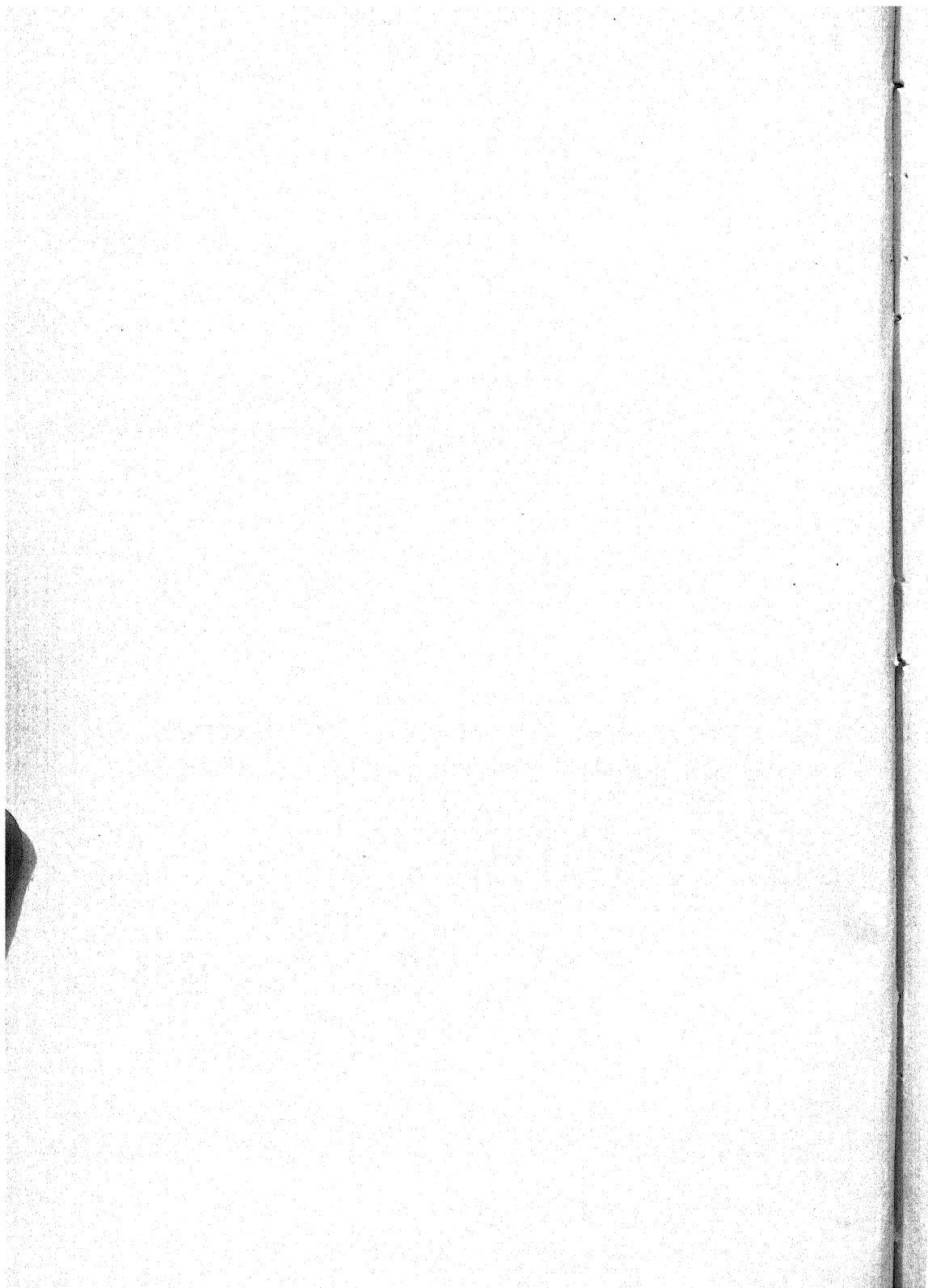
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THE CHAROPHYTES OF THE BOMBAY PRESIDENCY

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Received for publication on 19th January, 1935.

The author has previously recorded in this journal seven species of Charophytes from Salsette near Bombay in 1931 (3). The present paper deals with nine more species of these plants which have been collected mainly from the environs of Poona in the Deccan and a few places from Kathiawar in the North-West. They have been mostly indentified by Mr. G. O. Allen, I.C.S. and by the late Mr. James Groves of England to whom the writer is greatly indebted.

The Presidency of Bombay represents arbitrary political limits rather than a natural unit with uniform climatic and topographical conditions. It has five distinct botanical provinces as mentioned by Dr. W. Gray in his famous essay in the gazetteer (4). It begins with a desert at its North-West extremity and ends in a monsoon forest at its southern ends. The Charophytes are the ancient inhabitants of its southern parts, as their fossil remains are found in the Inter-trappean rocks of the Deccan Trap. One species has been described under the name of *Chara Malcolmsoni* (10).

No attempt has been made here either to describe or figure the well known species mentioned in the literature cited at the end.

I. NITELLA.

1. *Nitella mucronata* Miquel.

Found at Pashan near Poona in a slowly flowing canal in December 1930. The plants were heavily incrustated with lime.

Distribution:—India (Saharanpur (Allen); Kashmir (Mukerji); Rangoon (Pal).) Europe; N. America; and N.W. Africa.

2. *Nitella furcata* Ag. (= *N. Roxburghii* Br.)

Collected from a shallow pond near Bhayender (Salsette) in September 1931.

Distribution:—India (Saharanpur (Allen); Kashmir (Mukerji); Burma (Pal); Coromandel and Ceylon). Philippine Islands; N. Australia; Madagascar.

II. CHARA.

3. *Chara corallina* Willd.

Stem 1 mm. broad. Oogonia 1072 μ long, 720 μ broad, Antheridium 575 μ diam. Oospore 800 μ long, 480 μ broad, showing about seven ridges. The plants had lime incrustation in rings. They had also an epiphytic green alga, *Chaetophora elegans* Roth., and a number of *Hydra* and *Vorticella* on them.

The plant looks very much like a robust form of *C. succincta* mentioned in my paper on Salsette Charophyta (3), but there are frequent instances of antheridia in the axil of the stem-whorl and outside it in addition to oogonia; and both oogonia and antheridia occurring together at the same branchlet-nodes rules out the former species.

Collected from a large pond at Thana (Salsette) in February 1932.

Distribution:—India (Saharanpur (Allen); Burma (Pal).) Ceylon; Sumatra; Philippine Islands; N. Australia.

4. *Chara Braunii* Gmel. (= *C. coronata* Br.)

This plant has been collected from two different places, viz., (1) from a pond in Cutch half way between Mandavi and Bhuj in March 1928, (4) from a large, deep pit at Wanowri near Poona in April 1932.

Distribution:—India (Saharanpur (Allen). This plant ascends to a height of 6,000 ft. in the western Himalayas.) It is world wide in its distribution.

5. *Chara pashanii* sp. nov.

Monoecious. Stem slender, the internodes a little less than the length of the branchlets. Cortex entirely absent. Whorls of 8-10 branchlets. Stipulodes entirely absent. Branchlets incurved, of 4-5 segments, segments often swollen, the lower 1-2 short and, curved, the middle one elongated, the tip short and acute. Bract-cells suppressed. Bracteoles when present as long as oogonia and acute. Gametangia produced at two lower nodes. Oogonia mostly geminate or rarely 3 together. Oogonium c. 544 μ long, 425 μ broad; spiral cells showing about 8 convolutions; corona c. 85 μ high, 170 μ broad. Oospore black, 425 μ long, 289 μ broad, ellipsoidal showing about 7 ridges. Antheridium 198 μ diam.; rarely geminate or solitary. Plant delicate in appearance and heavily incrustated with lime.

A species of *Zygnema* was epiphytic on it. Collected from a ditch at Pashan near Poona in December 1930.

This plant comes very near *C. Braunii* but it has marked differences. The absence of stipulodes and swollen branchlet segments at once separates it from *C. Braunii*. This species has very few bract cells and they are very slender and absent from the upper nodes

so that there is no cluster at the tip of the branchlet characteristic of *C. Braunii*. The lowest branchlet segment is very much curved. The corona of the oogonium also differs in form. The plant is incrusted all over and not in rings.

There is no mention of rudimentary stipulodes in any form of *C. Braunii* mentioned by Nordstedt's Australasian Characeae (9) or Synopsis Characearum Europaeorum by Migula (7). Recently Mr. B. P. Pal (11) has described a new species *C. nuda* from Burma belonging to the ecorticate haplostephanous group of *Chara* but his plant has small and acute stipulodes, always solitary sex-organs and the size of the plant does not exceed six inches. The size of the oogonia is larger than that of the present species. The shape of the corona also differs. Therefore, this Pashan plant is quite distinct. (Fig. 1).

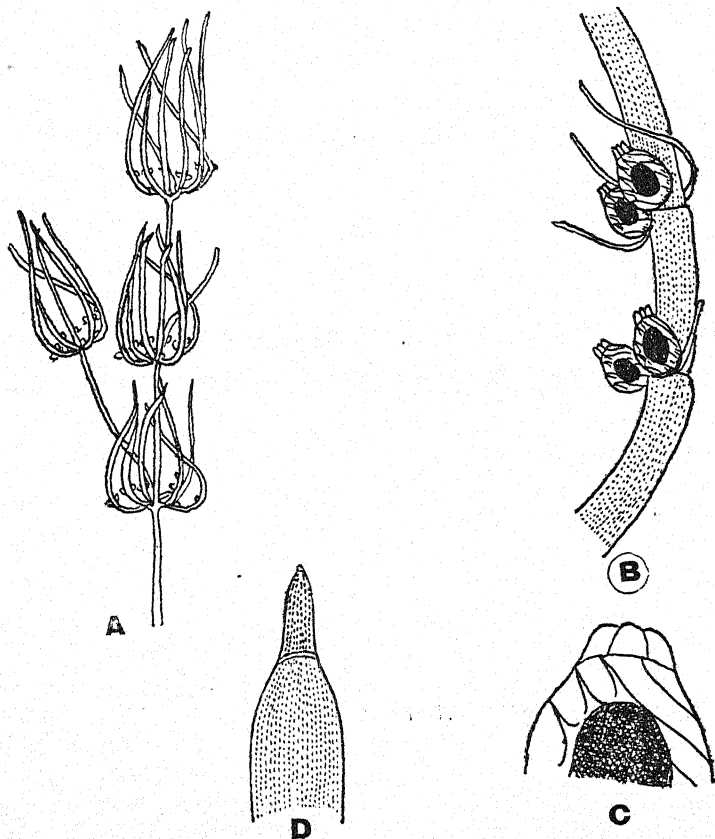


FIG. 1. *C. pashanii*. A Plant $\times c. 3$; B Branchlet-node with oogonia $\times c. 18$; C Corona of oogonium $\times c. 60$; D Apex of branchlet $\times c. 60$.

6. *Chara gymnophylla* Br.

Monoecious. Stem of medium thickness. Cortex diplostichous and regular. Spine-cells spherical and rudimentary. Branchlets 10 in a whorl; incurved; ecorticate and of six segments; as long as an internode. Basal segment rarely imperfectly corticate. Stipulodes rudimentary. Bract-cells rudimentary. Bracteoles varying in length—as long as a segment. Oogonia as many as 3-6 at a node.

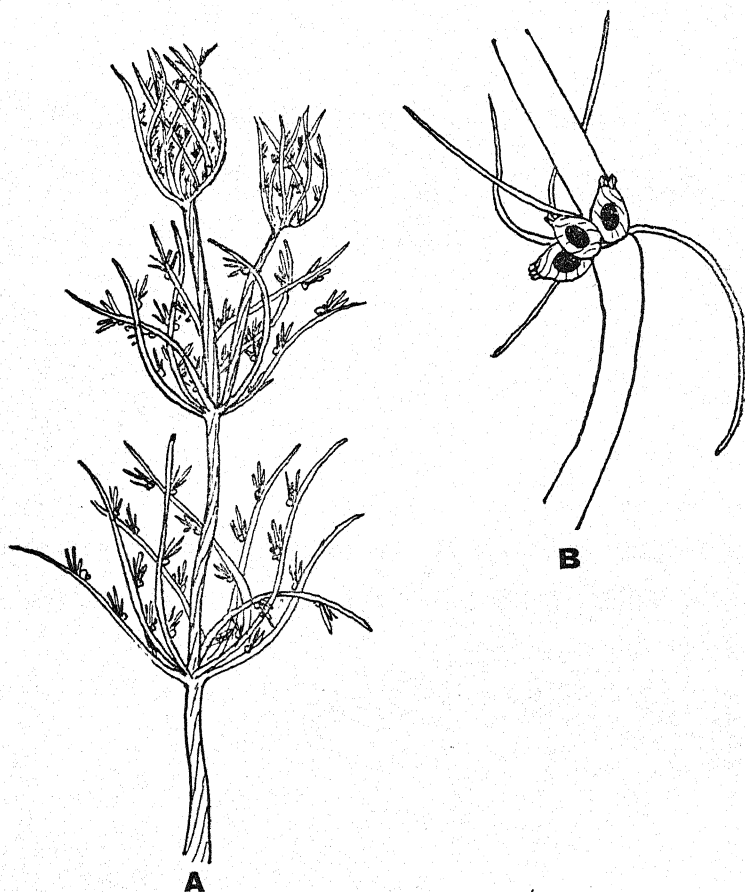


FIG. 2. *C. gymnophylla*. A Plant $\times c. 3$; B Branchlet-node with oogonia $\times c. 15$.

Gametangia usually at three nodes. Oogonium 560μ long; 420μ broad; spiral cells show 11-13 convolutions; corona 165μ broad; 70μ high. Antheridium c. 588μ diam.; 1-2 at a node. The plant is about 15 cm. long and heavily incrustated with lime. Staining with iodine revealed abundance of starch in every part including the antheridia.

Found abundantly in slow running water at Pashan and in the River Indrayani at Moshi near Poona in December 1930. (Fig. 2.)

This plant is almost identical with *C. gymnophylla* Br. (for which the earliest and therefore valid name seems to be *C. squamosa* Desf.) Braun in his later works regarded it as a sub-species of *C. vulgaris* (= *C. foetida* Br.). But the interesting and the important difference of the Poona plant is the production of several (3-6) oogonia at the same node. Mr. Pal (11) has described three new species of *Chara* with ecorticate branchlets but none of his species has this characteristic feature.

Distribution:—India (Groves); Burma (Pal.), Europe; Africa.

7. *Chara flaccida* Br.

Monoecious. Stem moderately stout and corticate. Spine-cells acuminate and well developed. Stipulodes in a single circle and quite prominent. Branchlets 8; ecorticate; segments 4-5. Bracts well developed. Bracteoles very much like bracts. Gametangia at lower three nodes. Oogonium 425 μ long; 289 μ broad; corona 153 μ broad; 68 μ high; spiral cells showing 8 convolutions. Antheridium 221 μ diam.; oospore golden brown. Plants moderately incrustated.

The general appearance of this plant is very much bristly and the stipulodes, bracts and bracteoles are very much alike in form and size. Except in the golden brown colour of its oospore it hardly differs from *C. gymnopitys*.

This plant was collected by Mr. S. Joshi, B.Sc., from a dry bed of the river Setrunjaya at Dhari (Kathiawar) in May 1933.

Distribution:—India (Salsette (Dixit); Kashmir (Mukerji); Burma (Pal).) Ceylon and tropics in general.

8. *Chara gymnopitys* Br.

Found in brackish water at Khar (Salsette) in January 1930.

Distribution:—India (Gonda (Allen); Burma (Pal).)

9. *Chara contraria* Kutz.

Specimens of this species were collected from two different places in Kathiawar, viz., (3) Barda-Hills in canals and (4) Jamnagar, in March 1929.

Distribution:—India (Saharanpur (Allen); Burma (Pal).) Europe; Africa and other continents.

10. *Chara fragilis* Desv.

This plant was collected from the river Indrayani at Moshi (Poona) in December 1931. A juvenile form of it was also found at Tilakwada (Baroda) from the bed of the river Narbada in October 1930.

The cortex in the Indrayani specimen was evenly triplostichous and the spine-cells and stipulodes were rudimentary.

Distribution:—India (Saharanpur (Allen); Pathankot-Punjab (Pande); Kashmir (Mukerji); Burma (Pal).) It is world wide in distribution being recorded from every country and clime; from ice cold waters as well as from hot springs.

11. *Chara zeylanica* Willd.

The lowest branchlet segment is commonly short but in this specimen it was rather long. This plant had a number of swollen stem-nodes giving out rhizoids and new shoots. It was collected from Katraj tank at Poona in April 1932.

Distribution:—India (Salsette (Dixit); Ahmedabad (Saxton in Flora N. Gujerat); Saharanpur (Allen); Burma (Pal); Kashmir (Mukerji).) Widely distributed in the tropics.

Summary

1. Eleven species of Charophytes mostly from Kathiawar and the Deccan are recorded.

2. Out of these eleven species two species of *Nitella* and seven species of *Chara* are newly recorded from the Presidency of Bombay.

3. A new species, *Chara pashanii*, and another interesting species, *Chara gymnophylla*, are described.

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ON THE OCCURRENCE OF GYMNOGRAMME CALOMELANOS KAULF. IN INDIA

BY

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Received for publication on 27th June, 1934.

With reference to the note by P. N. Mehra, in Vol. XI, No. 4 of this Journal, on *Ceropteris calomelanos* L. in Sikkim (which fern is obviously *Gymnogramme calomelanos* Kaulf.), it will interest many to know that this fern has already been described under the name of *Gymnogramme calomelanos* Kaulf. in "The Ferns of Bombay" by Blatter and d'Almeida* as far back as 1922. Specimens of the fern from Rowli Hill (Bombay Island) were exhibited at a meeting of the Bombay Natural History Society held on the 27th of February 1919 when the present writer read a paper on "The Wild Ferns of Bombay Island and Salsette." Prior to this date, it appears, there is no mention of the existence of this fern anywhere in India. It is evidently an exotic.

For the benefit of those who are interested in the spread and distribution of the fern in India the writer quotes below pertinent extracts from "The Ferns of Bombay":—

"Distribution: Bombay Presidency — Khandala and Kam-poli; Dango Forest (Surat); Bombay Island, Malabar Hill, Sewrie, Rowli Hill, Sion Wood.—Nilgiris; Ceylon.

"It is a native of the West Indies, Jamaica, St. Domingo, Guiana, Brazil, Mexico, the Island of St. Catherine, the Carriba Islands, etc."

"It propagates freely by spores and this probably accounts for its having run wild in and about Bombay. It is particularly abundant on the hill sides which have been cut down for reclamation purposes. It prefers a bright open situation as is evidenced by its greater abundance on the sunny hill sides at Rowli than in the shade of the Sion Wood. The hill sides also allow of free drainage without which the fern cannot thrive."

* "The Ferns of Bombay" by E. Blatter and J. F. d'Almeida, Bombay, 1922.

On account of its ready propagation from spores it is one of the most easily grown ferns and has, therefore, of late years, found its way into many of the Bombay gardens.

Since the publication of "The Ferns of Bombay" the writer has also seen this fern growing in large numbers on the southern slope of Mount Mary, Bandra (Salsette Island) overlooking the Mahim Creek and within a few yards of the beach, where it is fully exposed to the prevalent winds and to the salt air from the sea. It will be of interest to note that there too, as at Rowli, the hill side has been quarried for reclamation purposes.

There is no doubt that, though an exotic, the fern has come to stay in India.

REVIEWS

SMITH, G. M., OVERTON, J. B., GILBERT, E. M., DENNISTON, R. H., BRYAN, G. S. and ALLEN, C. E.—A Text Book of General Botany. Third Edition, pp. 574, 429 figs. Macmillan Co., New York, 1935.

In the six years that have elapsed since the publication of the last edition of the well known Wisconsin text-book, it has had such a favourable reception, that a revised edition is heartily welcomed. Even the first edition had fewer defects than several other text-books that have been published during the last decade. The increase of 35 pages of useful matter and the addition of several new illustrations is a decided improvement on what was already good for elementary students. The treatment of reduction division is adequate and it is refreshing to find that references have been made to several recent discoveries in such a simple way that even the elementary student can understand them. Special mention may be made of the work on heterothallism in fungi and the behaviour of chromonemata in mitosis.

The usefulness of the book could be still further increased by the addition of a full chapter on control of plant diseases and giving more details on pollination. In the life history of *Pinus* it is necessary to indicate the time taken by the development of the male and female gametophytes and the formation of seed. Some touches of human interest would enliven the subject and the addition of a few more photographs of prominent botanists would help to inspire the youthful mind. None of these defects, however, is serious enough to detract from the value of the book and space for any further additional matter should now be made only by cutting down some other parts here and there.

P. MAHESHWARI.

DAHLGREN, K. V. O.—Die Embryologie von *Impatiens Roylei*. Svensk Bot. Tidskr. 28: pp. 103-125. 1934.

The question of the origin of haustoria in the ovules of *Impatiens* has engaged the attention of several embryologists and different interpretations have been offered. The most recent contribution on the subject is by Dr. K. V. O. Dahlgren of Uppsala, who has given a clear and well-illustrated account based on the study of *I. Roylei* Walp. (= *I. glanduligera* Royl.).

The nucellus is long and narrow and has a hypodermal archesporial cell which functions directly as the megaspore mother cell. The embryo-sac is of the usual 8-nucleate type, but develops

according to the *Scilla*-scheme. There are two integuments of which the inner forms an integumentary tapetum round the embryo-sac. The antipodal cells are ephemeral. The polar nuclei fuse in the upper part of the embryo-sac and the primary endosperm nucleus divides to form a large chalazal and a small micropylar chamber. Free nuclear divisions occur in the former, while the latter divides transversely to form three cells. Of these the uppermost gives rise to a large haustorium which sends out branches into the funiculus, the second forms a tissue of cells surrounding the young embryo and the third has free endosperm nuclei. The line of separation between this last cell and the primary chalazal chamber generally vanishes. Now the endosperm becomes cellular at the periphery and the cells adjacent to the embryo become richly protoplasmic. It is noteworthy that in older ovules globular masses of plasma, with several endosperm nuclei imbedded within them, often get isolated and may be seen floating in the central vacuole, just below the tips of the cotyledons. Such isolated groups of "vesicles" have also been seen in the ovules of *Musa* by Juliano and Alcalá (1933).

P. MAHESHWARI.

The Journal of the Indian Botanical Society

(Formerly "The Journal of Indian Botany")

VOL. XIV

OCTOBER, 1935

No. 4

CARBOHYDRATE/NITROGEN RATIO OF THE SHOOTS OF SOME TROPICAL TREES

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Received for publication on 5th September, 1934.

Introduction

The question of development of flowers and fruits is of great importance and it is known that their formation occurs only under definite conditions. Klebs (1918) drew some important conclusions about the condition which favour vegetative and reproductive growth and he correlated them with the carbon-dioxide assimilation, absorption of water and salts and the intensity of light of temperature. The influence of light on the inception of reproductive phase is very well known. Garner and Allard (1920) have by proper adjustment of exposure to light converted annuals into biennials and biennials were made to flower in a few months. They have also shown by exposing the stem and branches of *Cosmos sulphureus* for different periods, that flowers can be produced on one part earlier than on the other region of the same plant.

The effect of duration of light on the behaviour of plant is studied from a chemical point of view by Pfeffer (1926) and Tinker (1928) and the main conclusion drawn is that the duration of light affects the carbohydrate/nitrogen ratio which has a great effect on the reproductive growth of the plants.

The amount of vegetative growth and the amount of reproductive growth made by a plant depends, according to the work of Kraus and Kraybill (1918), on the relative quantities of carbohydrates and nitrogenous substances which are available. A low carbohydrate/nitrogen ratio results in vegetative growth while a high rate reduces the vegetative growth without inducing reproductive growth. An intermediate ratio is best for vigorous vegetative and reproductive growth. The implications of these results for horticultural practice are great and many workers have followed up this line of investigation and have tried to correlate vegetativeness and fruitfulness with the carbohydrate/nitrogen ratios. These observations of Kraus and Kraybill were extended by workers like Woo (1919) who has shown that the different range of carbohydrate/nitrogen ratio is required to produce the same range of effect in different plants. Gurjar (1920) showed in the case of the tomato that the carbohydrate/nitrogen ratio may vary between 2 and 19 but the best fruiting occurs only when the ratio is between 4 and 6.

The application of Kraus and Kraybill's method to apples is of special interest as it has a biennial fruit-bearing habit. Hooker (1922) has shown that the decrease in carbohydrate/nitrogen ratio produces vegetative growth while a high ratio results in the formation of fruit buds. If the spurs are defoliated, as shown by Harvey and Murneek (1921), in summer of the first year before the fruit buds are formed there is a lessened amount of fruit bud formation due to the alteration of carbohydrate/nitrogen ratio as the carbohydrates are not synthesised. Similar results are also obtained by Roberts (1921).

The above short review of the work done on the physiology of reproduction in flowering plants show that the plants selected for the experimental work are mostly cultivated plants. No attempt is made to study the causes underlying the production of flowers in wild plants growing in natural conditions or why these different plants behave in different manner. It is a common knowledge that the development of flowers and foliage occurs at different times and they are associated with different seasons of the year. Some plants produce flowers throughout the year, some once or many times in a year and some once in several years. The factors governing the production of flowers in these different types of plants must be different and it is necessary to understand why certain plants come in the reproductive phase once in several years and others several times or once in a year. Tropical trees furnish an excellent material for such a study. It is known that when the flowering depends upon the season, the flowers are produced in some trees when they are entirely leafless. In these trees the vegetative growth stops before the reproductive growth occurs. The causes underlying this rather unusual behaviour of plants are obscure and it is not known whether these causes are external or internal or both.

The Monsoon forests near Bombay are composed of trees which exhibit such behaviour with some minor differences and these trees afford good material for studying this aspect of the physiology of reproduction as it occurs in nature. No attempt is made to study the causes of such behaviour and it is here attempted to make a beginning in that direction, though the difficulties of working with the trees under natural conditions are enormous and it is not possible to obtain scientific results of great accuracy.

Generally the leaves are shed by plants after the flowers and fruits are produced and remain in the leafless condition till the beginning of the vegetative activity in the next season. But in the case of these deciduous trees the leaves are shed long before the flowers are produced. It is an unusual sight to see a leafless tree bearing flowers. The shedding of the leaves generally occurs at the end of the wet season and it is possible that the scarcity of water in the soil may be responsible for their fall. But this does not appear to be the reason as the small plants grown in the pots and watered regularly shed their leaves as they did in nature.

Investigation

In the beginning the survey of trees growing in the Monsoon forest near Bombay was undertaken for the determination and consequent selection of the trees for this investigation. It was found that though there are great variations in the inception of reproductive phase as related to the vegetative phase in the trees growing in these forests, these variations can be roughly classified as follows:—

- (1) Trees shedding their leaves long before the flowers are produced. The flowers produced in the leafless condition of the trees.
- (2) Trees shedding their leaves and then producing flowers and leaves together at the same time.
- (3) Trees shedding their leaves and producing fresh leaves before flowers are produced.

The leaves are shed at different times but during the dry months from October to May. The shedding may occur twice during the dry months.

It was first undertaken to study the trees belonging to the class (1) mentioned above and it consists of trees which have their reproductive growth in leafless or almost leafless condition of the trees, *i.e.*, when the vegetative activity is entirely suppressed.

Bombax malabaricum DC. offers a clear case in point. The vegetative activity which manifests itself by the production of new foliage begins to appear in the month of May or even earlier, indicating that the initiation of vegetative activity is not dependent on the return of wet weather as the monsoon does not set in till the first half of June. The new leaves continue to be produced until November even when some of the leaves produced earlier are being

shed. The leaves produced later remain very small and drop off a short time after they are produced. So there is a gradual slackening of the vegetative activity from October onwards. There is a certain amount of variation in detail for each tree but these statements indicate the general behaviour of the species.

The flowers begin to appear from January and the trees are studded with flowers in the month of February. The flowering activity lasts for nearly two months, though the actual period of flowering may vary from tree to tree, some beginning to flower as early as December and some as late as February.

The flower buds always appear on the shoots, which had the leaves during the last vegetative activity (*i.e.*, May to November) and in the axils of the leaves which are shed, *i.e.*, above the scars left by the shed leaves. When the next vegetative activity begins the branch will elongate and produce fresh leaves or a new shoot may develop as a bud on the last year's shoot or on a portion of a branch two years old.

Another tree selected is *Cassia fistula* Linn. The vegetative activity begins in May or even later after the Monsoon has set in. It may begin even in April, if the flowering activity is suppressed. The new leaves are being produced till the end of the Monsoon, *i.e.*, till October. The leaflets begin to drop off from February and the shedding may continue till the middle of April. The time of shedding leaves fluctuates from middle of February to middle of April. The flowering activity commences soon after the leaves are shed. The flowering activity continues for two months in all the cases. The third tree selected is *Cassia renigera* Wall. If the flowering activity is normal the vegetative activity begins in May but it may develop new leaves in April, if the flowering activity is nil or below normal. The shedding of the leaves begins in Bombay in February and the tree becomes leafless in the middle of April. Flowering sets in as soon as the tree is leafless or slightly before the tree has shed all the leaves. The flowering period is of two months from April to the end of May but it may vary according to the climatic conditions.

The fourth tree selected is *Poinciana regia* Bojer. which is not an indigenous tree. The vegetative activity continues all through the year, though it is very much suppressed in the months of March and April. If the flowers are produced the vegetative activity stops. In the latter case the leaves begin to be shed in March till April. The vegetative activity begins from June and lasts till February. The flowering commences by the middle of April and continues up to the end of May, varying with individual tree.

The above description of four trees and their periods of vegetative and reproductive growth make it clear that in *Bombax malabaricum* DC. the two activities of the tree are separated by an interval while in other cases there is no such interval of time between the reproductive and vegetative activity. In the absence

of the flowering phase the vegetative growth may commence earlier than usual. These points are of importance when taking samples of shoots either for physiological experiments or for chemical analysis. If the attention is not paid to the above-mentioned facts, it is likely that unreliable and complex results are obtained. For the elucidation of this alternate inception of activity sharply marked off in time it is necessary to bear the above facts in mind and to select samples from the trees showing normal activity.

It is evident from the account given above about the vegetative and reproductive growth of these trees that the same shoot bears the flowers after the leaves are shed. It is therefore necessary to study the carbohydrate/nitrogen ratio of the shoots of these trees and to determine if the vegetative and reproductive growth can be correlated to the differences in the carbohydrate/nitrogen ratios during the two phases.

Anatomical Features

Before investigating the C/N ratios of these trees it was considered necessary to study the anatomical features of the shoots during the vegetative and flowering phases. The anatomy of the shoots bearing leaves and flowers was studied. In the case of *Bombax malabaricum* DC. the development of the wood is very little and the secondary growth in thickness due to the activity of cambium is negligible during the first year. A large amount of phloem tissue is developed as a result of the cambium activity. The cambium activity ceases at the time of leaf fall and commences again when the vegetative growth begins next year. During the reproductive phase there is no formation of conducting elements as the cambium activity is suppressed. As soon as the leafy buds begin to develop in the month of May or June the cambium cells begin to divide and secondary xylem and phloem are produced in the second year shoots. It indicates that the development of the leaves is accompanied by the development of the conducting tissue. The amount of secondary phloem developed is always in great excess of the secondary xylem and as a result of this unequal development of the two regions the trees are soft wooded.

Shoots of *Cassia renigera* Wall, *Cassia fistula* Linn. and *Poinciana regia* Bojer. were similarly studied and in all these trees the cambium remains active for longer period during the year than in the case of shoots of *Bombax malabaricum* DC. The cambium becomes inactive only during the time when the leaves are shed. The interval between the leafless condition and the next vegetative phase is so small that it is difficult to determine the period of the meristematic inactivity.

There is a greater production of secondary xylem than that of the secondary phloem and in that respect these trees differ from *Bombax malabaricum* DC. The shoots of these trees were also

examined when they were bearing flowers and no differences in the anatomical features were noticed.

Carbohydrate / Nitrogen Ratio

It is necessary to determine the total carbohydrate contents and the total nitrogen of the new shoots beginning from the vegetative activity upto the termination of the flowering phase. In order to get complete data it was undertaken to determine the carbohydrate contents of the new shoots (first year) every month and in order to get comparable data it is necessary to make careful selection of shoots for analysis. If the first year shoots are not taken, all determinations and results are likely to be complex and unreliable. In some trees like *Bombax malabaricum* DC. it is not possible to determine the base of the first year shoot as some of the lower leaves drop during the current vegetative season and therefore selection becomes difficult. In some cases the stages of the shoots selected during pre-flowering stage may not be normal and would not have produced flowers if they had been allowed to remain on the tree. Such abnormalities are not uncommon in these trees and the errors are consequently unavoidable. These trees are very high and there is some difficulty in reaching for the proper shoots. In order to minimise the error a large number of normal looking first year shoots are taken and cut into minute bits and mixed up and a known weight taken for analysis. The difficulties of selection are great when the shoots are in leafless condition and it is not possible to say whether they are normal or not, or whether they are first year or second year shoots. There is another source of error. The new shoots continue to be formed for several months at least from June to October in *Bombax malabaricum* DC. and even later in other trees. So the selection of shoots is rendered difficult as it is necessary to select the shoots produced at the same time on the plant and analyse those during the year so as to get the idea of the sequence of carbohydrate and nitrogen changes in the shoots during the year. If in the month of August or September a shoot which is not developed in the month of June but later, is selected, it will give an inaccurate and non-comparable results. This difficulty is avoided either by noting the scars of the freshly fallen leaves and making proper marks in June for the shoots of the similar growth to be selected for analysis later.

Selection of Material

Branches weighing about 100 gms. were cut to small pieces and 25 gms. of material was carefully weighed and put up for drying. After the material is dried, it is powdered in a grinder and carefully weighed. From the remaining cut material a weighed quantity was also used for extraction of carbohydrates.

The method for extracting carbohydrates worked out by Davis and Daish (1916) was adopted mainly in this investigation. The

sugars and starch are determined according to the method of Dastur and Samant (1933).

Kjeldahl's method, further modified by Gunning (1930), was used for estimation of total nitrogen.

***Bombax malabaricum* DC.**

The shoots were selected for analysis from the same tree during the year. The leaf-fall started at the beginning of January though the formation of new shoots stopped at the end of September. In November the leaves lost their shining appearance and appeared yellowish-green. The January reading includes the leafless shoots while those of December and November had leaves, but they dropped as soon as the samples were brought to the laboratory. The shoots analysed in February, March and April bore flowers. In April the fruit formation had already commenced. The shoots selected in May had produced fruits which were dropped.

The following Table I gives the results of the carbohydrate analysis and the total nitrogen of the first year shoots during the months of June to May. It will be seen that the total sugars as hexoses are high during the early vegetative phase in the month of July and August and in the flowering phase during the months of February, March and April. The high value of sugars during July and August may be due to the downward flow of sugar from the leaves on account of the photosynthetic activity. The photosynthetic activity becomes low in the month of October and consequently the sugar contents of the shoots are low in the months of October to January. When the flowering activity commences there is an upward flow of carbohydrates and consequently the sugar contents of the shoots again become higher and remain high till the flowering activity ends. In May the sugar contents are very low when there is neither flowering activity nor vegetative growth. Similarly starch contents of the shoots are highest during active vegetative phase on account of the photosynthetic activity when sugars are converted into starch. The starch contents are not equally high during the flowering stages as the sugars are directly utilised for the formation of flowers and fruits. The carbohydrate contents are consequently highest during the active vegetative and flowering phase.

These results show that there is downward flow of carbohydrate materials formed in the leaves and consequently the carbohydrate contents are higher in the shoots. These higher contents of carbohydrates may alter the C/N ratios and may cause increased vegetative growth till they pass down to lower regions. During the reproductive phase the flow of carbohydrates is in the upward direction from the portion below where they are temporarily stored and they are utilised for the purpose of reproductive growth.

The nitrogen contents fluctuate between 0.1779 and 0.2875. The contents of the shoots are higher during the reproductive and

TABLE I

Carbohydrate Contents and Total Nitrogen of the Shoots of *Bombax malabaricum* DC. during the Vegetative and Reproductive Phases in Gms. per 100 Gms. of Fresh Material

Date.	The phase of growth.	Total sugars as hexoses in gms.	Total starch as hexoses in gms.	Total carbohydrates as hexoses in gms.	Total nitrogen in gms.
2nd June.	New shoots with leaves	0.0795	0.0265	0.1060	0.2441
7th July.	Leafy shoots	0.7590	0.1133	0.8728	0.2875
5th Aug.	Do.	1.1040	0.2274	1.3314	0.2825
10th Oct.	Do.	0.2688	0.0298	0.2986	0.1779
5th Nov.	Leaf-fall begins	0.1851	0.0364	0.2215	0.1835
8th Dec.	Leaf-fall continues	..	0.0971	0.0971	0.1782
3rd Jan.	Leafless	0.0615	0.0490	0.1105	0.1890
9th Feb.	Shoots with small flower buds	0.9410	0.0702	1.0112	0.1935
2nd Mar.	Flowering shoots	0.8334	0.0728	0.9062	0.2550
1st Apr.	Flowering shoots fruits formed	0.7233	0.0993	0.8226	0.2506
3rd May.	Flowering ends and fruits dropped	0.0242	0.0191	0.0433	0.2389

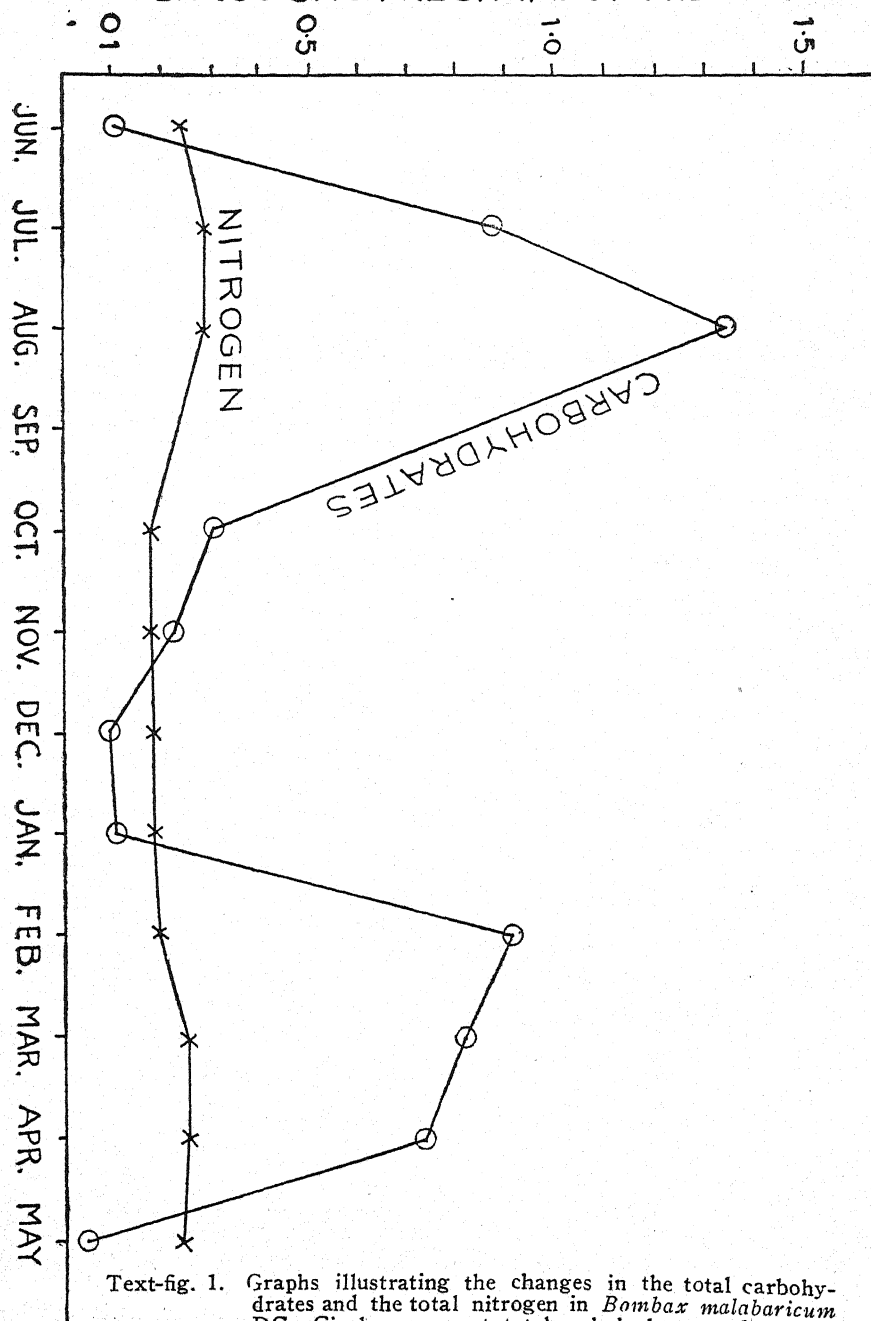
vegetative phases than those of the shoots during the other months in the year.

The graphs illustrating the changes in the carbohydrate contents and the total nitrogen in the shoots of *Bombax malabaricum* DC. are given in Text-figure 1.

The two species of *Cassia*

The medium sized shoots of the current year were selected for analysis from branches at a height of about 10 to 15 feet. The leaf-fall began in February, though the drying up of leaves began as

GMS. PER 100 GMS. FRESH WT. OF THE SHOOTS



Text-fig. 1. Graphs illustrating the changes in the total carbohydrates and the total nitrogen in *Bombax malabaricum* DC. Circles represent total carbohydrates and crosses represent total nitrogen.

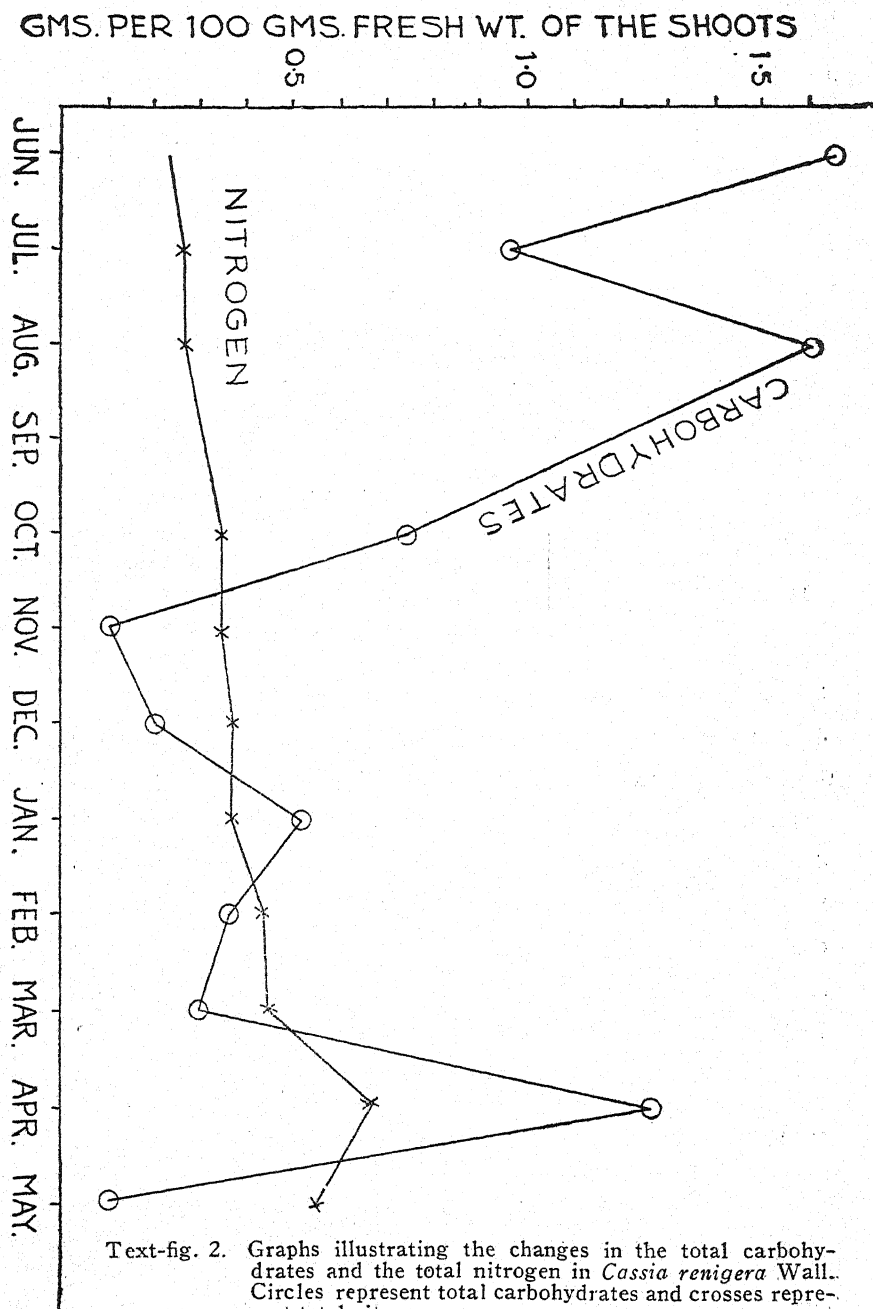
early as November. In March the leafless shoots were taken for analysis, in April shoots bearing flower buds were analysed and in May the shoots which had dropped their flowers were analysed. *In all the cases only the branch stem portions were analysed,* the leaves or flowers being always removed before the shoots were weighed. The results of analyses are given in the Tables II and III for *Cassia renigera* Wall. and *Cassia fistula* Linn. respectively.

TABLE II

Carbohydrate Contents and Total Nitrogen of the Shoots of *Cassia renigera* Wall. during the Vegetative and Reproductive Phases in Gms. per 100 Gms. of Fresh Material.

Date.	The phase of growth.	Total sugars as hexoses in gms.	Total starch as hexoses in gms.	Total carbohydrates as hexoses in gms.	Total nitrogen in gms.
7th June	New shoots with leaves	1.5620	0.0833	1.6453	0.2295
6th July	Do.	0.8368	0.1093	0.9461	0.2570
11th Aug.	Do.	1.5030	0.0920	1.5950	0.2599
2nd Oct.	Do.	0.6733	0.0550	0.7283	0.3397
3rd Nov.	Leaves showing signs of drying up	0.0500	0.0457	0.0957	0.3339
5th Dec.	Do.	0.0313	0.1690	0.2003	0.3600
7th Jan.	Do.	0.4255	0.0800	0.5055	0.3650
2nd Feb.	Leaf-fall begins	0.2057	0.1450	0.3507	0.4332
1st Mar.	Leafless shoots	0.0944	0.1944	0.2888	0.4350
4th Apr.	Shoots with small flower buds	1.1860	0.0642	1.2502	0.6600
10th May	Flowering ends, flowers dropped	0.0517	0.0593	0.1110	0.5410

As in the case of *Bombax malabaricum* DC. the shoots of the two species of *Cassia* contain largest amount of carbohydrates during the active vegetative growth during the months of June, July and August and very probably in September as the values of carbohydrates are also pretty high in October. Both the sugar contents



and the starch contents are the highest on account of the photosynthetic activity. There is a fall of carbohydrate contents in the months of November or December with a rise in December or January. In January the trees pass through a short period of vegetative activity. When the leaf-fall begins the carbohydrate contents become low. In the flowering stage the carbohydrate contents again reach a second maximum indicating an upward gradient in the carbohydrate flow (Text-figs. 2 and 3). The nitrogen contents of the shoots rise continuously from June upwards and the highest nitrogen contents are found in shoots at the time of flowering after which it declines (Text-figs. 2 and 3). These changes in total nitrogen are different from those found in *Bombax malabaricum* DC.

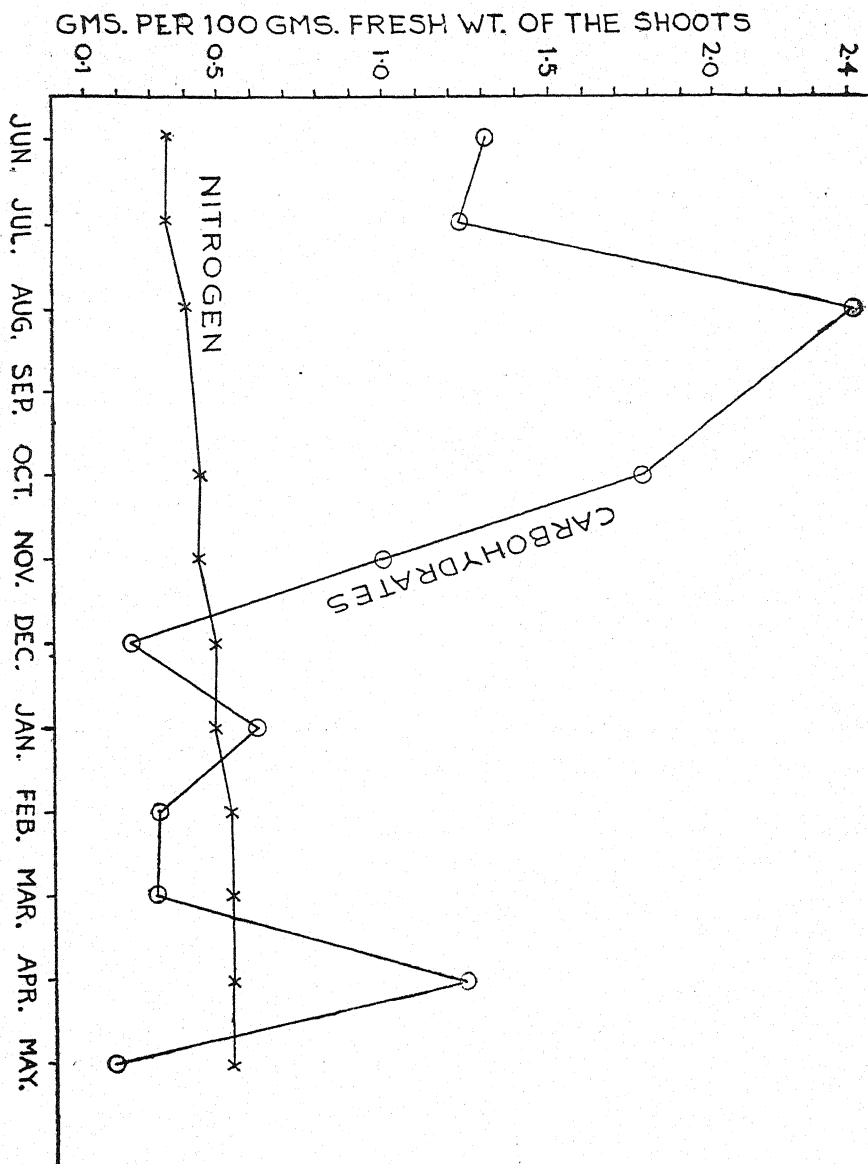
TABLE III

Carbohydrate and Total Nitrogen Contents of the Shoots of *Cassia fistula* Linn. during the Vegetative and Reproductive Phases in Gms. per 100 gms. of Fresh Material

Date.	The phase of growth.	Total sugars as hexoses in gms.	Total starch as hexoses in gms.	Total carbohydrates as hexoses in gms.	Total nitrogen in gms.
8th June	New shoots with leaves	1.1940	0.1194	1.3134	0.3503
6th July	Do.	1.1060	0.1254	1.2314	0.3406
10th Aug.	Do.	2.2870	0.1314	2.4184	0.4056
3rd Oct.	Do.	1.7780	0.0070	1.7850	0.4413
4th Nov.	Leaves begin to dry up	1.8960	0.1007	0.9987	0.4325
5th Dec.	Do.	0.0183	0.2291	0.2474	0.4941
6th Jan.	Do.	0.5546	0.0623	0.6169	0.4890
3rd Feb.	Leaf-fall begins	0.0975	0.2191	0.3166	0.5434
1st Mar.	Leafless shoots	0.1350	0.1787	0.3137	0.5431
3rd Apr.	Flowering shoots with small buds	1.0650	0.1893	1.2543	0.5510
9th May	Flowers dropped.	0.0937	0.0883	0.1820	0.5423

***Poinciana regia* Bojer.**

The task of selecting branches is rendered extremely difficult as the vegetative growth continues till February and is very irregular. The growth is vigorous in one month and slackens off in the next.



Text-fig. 3. Graphs illustrating the changes in the total carbohydrates and the total nitrogen in *Cassia fistula* Linn. Circles represent total carbohydrates and crosses represent total nitrogen.

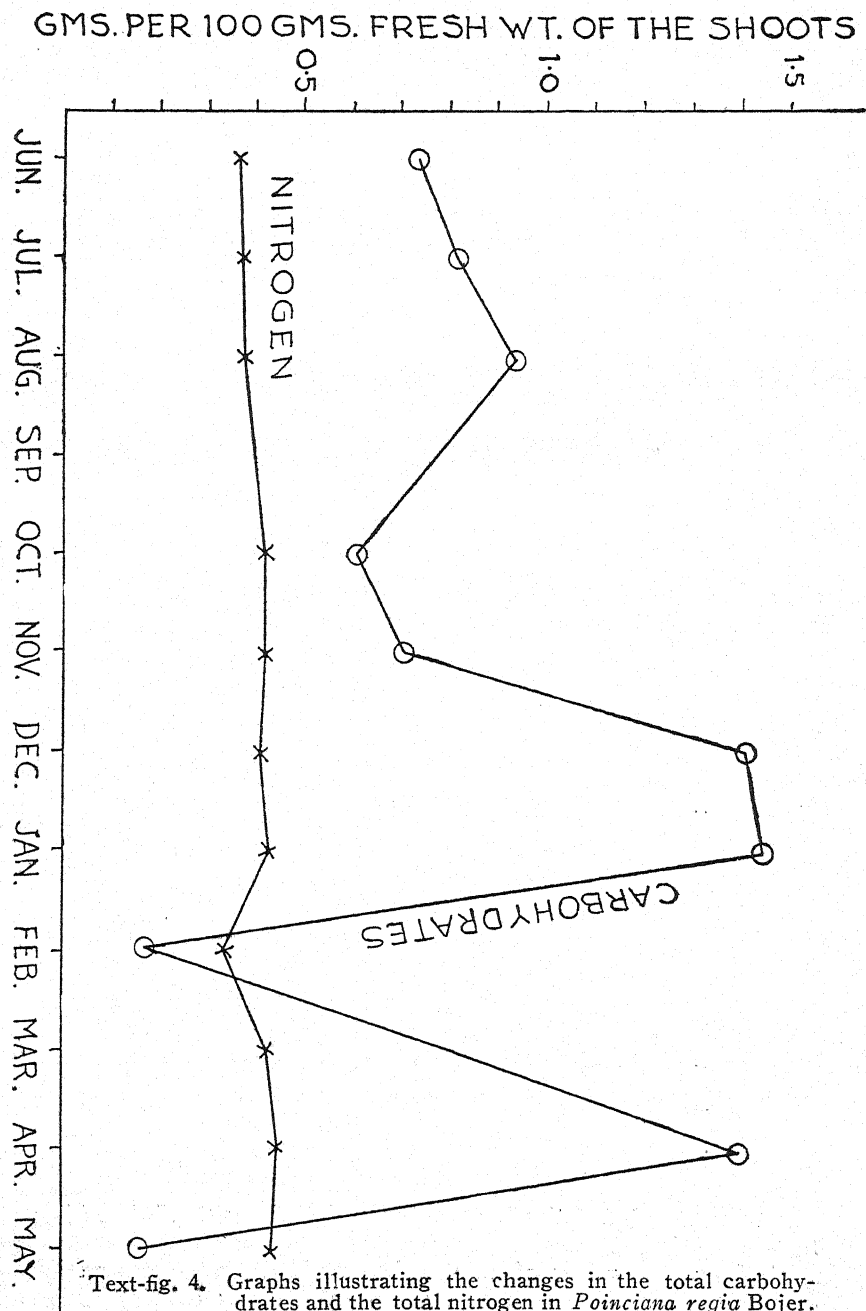
The vegetative growth was feeble in October and November and vigorous growth occurred in December and January. As there is

apical growth of the shoots, difficulty is experienced in distinguishing the shoots of the current year from that of the previous year. This irregularity of growth is borne out in the results of the analysis given in the Table IV. The leaf-fall began late in February. In March, April and May leafless shoots, shoots bearing flowers and the shoots with flowers dropped are respectively selected for analysis as in the case of the two species of *Cassia*.

Total sugar contents and carbohydrate contents are highest in the shoots analysed in December and January and very probably due to second period of maximum photosynthetic activity (Table IV and Text-fig. 4). Similar indications of greater assimilatory activity in January are also seen in the case of two species of *Cassia* (*vide* Tables II and III). The total carbohydrates are again very high in the flowering stage.

TABLE IV
Carbohydrate and Total Nitrogen Contents of the
Shoots of *Poinciana regia* Bojer. during the
Vegetative and Reproductive Phases in gms.
per 100 gms. of Fresh Material.

Date.	The phase of growth.	Total sugars as hexoses in gms.	Total starch as hexoses in gms.	Total carbohydrates as hexoses in gms.	Total nitrogen in gms.
4th June	New shoots with leaves	0.6206	0.1092	0.7298	0.3579
5th July	Do.	0.7169	0.0901	0.8070	0.3612
7th Aug.	Do.	0.8356	0.0852	0.9208	0.3640
2nd Oct.	Do.	0.5815	0.0212	0.6027	0.4119
6th Nov..	Do.	0.6739	0.0254	0.6993	0.4175
3rd Dec.	Vigorous shoots	1.2880	0.1219	1.4099	0.4020
6th Jan.	Do.	1.3390	0.0993	1.4383	0.4235
5th Feb.	Leaf-fall begins	0.0602	0.1015	0.1617	0.3255
1st Mar.	Leafless shoots	0.5745	0.2161	0.7906	0.4190
9th Apr.	Flowering shoots	1.2500	0.1302	1.3802	0.4427
12th May	Flowers dropped	0.0733	0.0781	0.1514	0.4345



Text-fig. 4. Graphs illustrating the changes in the total carbohydrates and the total nitrogen in *Poinciana regia* Bojer. Circles represent total carbohydrates and crosses represent total nitrogen.

Total nitrogen contents of the shoots increase from June to May as in the case of the two *Cassia* species (Table IV and Text-fig. 4).

The Carbohydrate/Nitrogen Ratios of the Trees

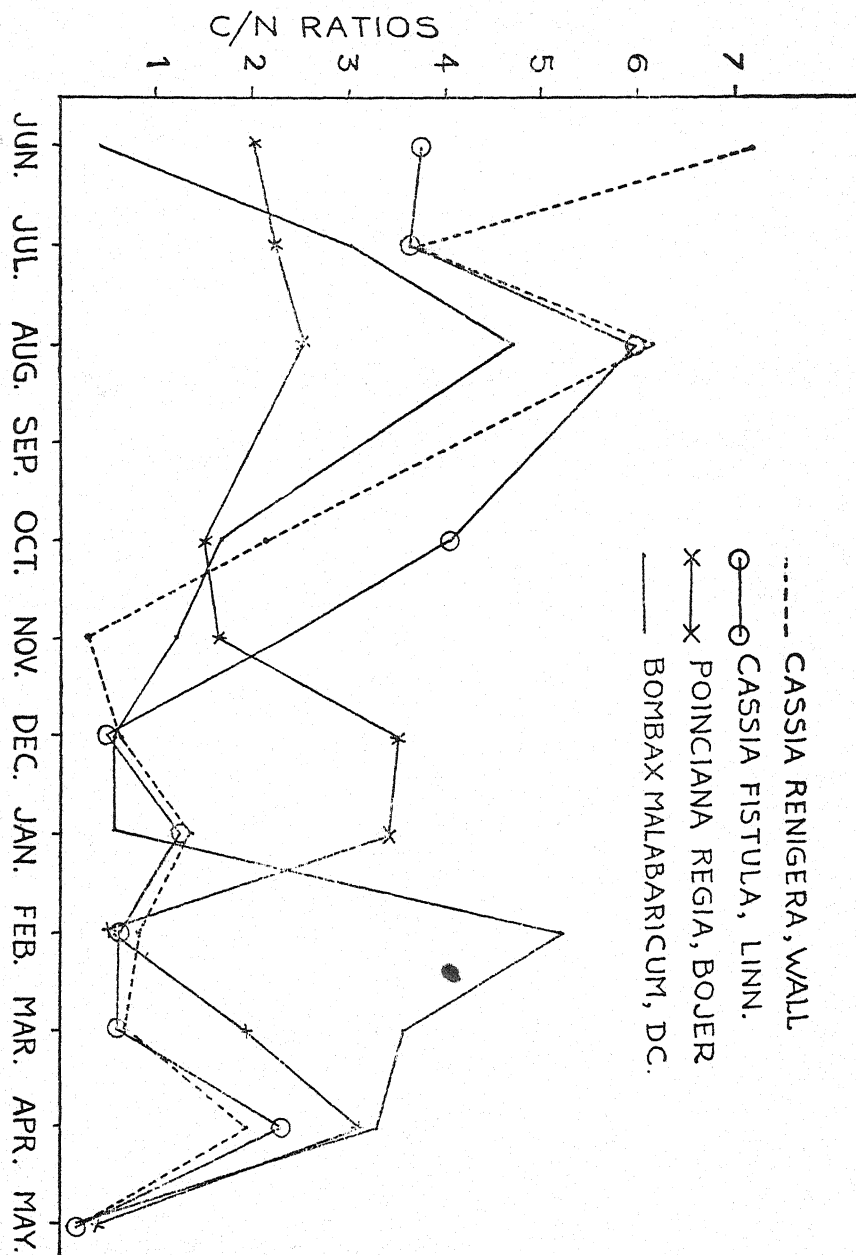
Having determined the total carbohydrate contents and the total nitrogen of the shoots of the current year every month, the C/N ratios are calculated to determine the fluctuations of the ratios during the vegetative and reproductive phases of the trees (Table V).

TABLE V

Carbohydrate/Nitrogen Ratios in the Shoots of *Bombax malabaricum* DC., *Cassia renigera* Wall., *Cassia fistula* Linn., and *Poinciana regia* Bojer. during the Vegetative and Reproductive Phases.

Date.	Carbohydrate/Nitrogen Ratios.			
	<i>Bombax malabaricum</i> DC.	<i>Cassia renigera</i> Wall.	<i>Cassia fistula</i> Linn.	<i>Poinciana regia</i> Bojer.
June ..	0.43	7.16	3.75	2.04
July ..	3.03	3.68	3.61	2.23
Aug. ..	4.71	6.14	5.96	2.53
Oct. ..	1.67	2.14	4.04	1.47
Nov. ..	1.21	0.29	2.31	1.67
Dec. ..	0.54	0.55	0.50	3.51
Jan. ..	0.58	1.38	1.26	3.40
Feb. ..	5.21	0.81	0.58	0.50
March ..	3.55	0.66	0.58	1.89
April ..	3.28	1.89	2.28	3.12
May ..	0.18	0.21	0.34	0.35

From the C/N ratios for *Bombax malabaricum* DC. it is evident that the periods of vegetative activity and reproductive activity are marked by high values of C/N ratios, while after the termination of vegetative and reproductive growth the values of C/N ratios are very low. Before the vegetative activity commenced the C/N



Text-fig 5. Graphs illustrating the changes in the Carbohydrate/Nitrogen ratios in the four trees. Circles represent total carbohydrates and crosses represent total nitrogen.

ratio is extremely low, viz., 0.18 and with the beginning of the vegetative growth it goes upto 0.43, (ultimately reaching the value of 4.7 in August when the vegetative growth is most vigorous. The C/N ratio falls to 1.67 in October and that marks the beginning of the termination of the vegetative growth. The C/N ratio reaches the value of 0.54 in December. Before the commencement of the flowering activity the C/N ratio is 0.58 and at the beginning of the flowering phase it reaches a high value of 5.21 (Text-fig. 5). The C/N ratio is higher at the inception of the flowering phase than the C/N ratio at the inception of vegetative phase.

In the case of the two species of *Cassia* the vegetative growth is marked with the highest values of the C/N ratios and the termination of the vegetative growth is marked with low values of the C/N ratios. Reproductive phase is set when the C/N ratio is medium. At the termination of the reproductive phase C/N ratio is lowest as in the case of *Bombax malabaricum* DC. (Text-fig. 5).

In *Poinciana regia* Bojer. the vegetative phase is marked by the C/N ratios varying between 2 and 3.5. The C/N ratio is markedly high when the vegetative growth becomes vigorous in December and January while the C/N is low in the months of February and March. At the reproductive phase the C/N ratio is again very high (Table V and Text-fig. 5).

Discussion

The anatomical investigation showed that the vegetative activity is accompanied by the cambium activity in the shoots and the latter is totally suppressed at the termination of the vegetative growth. There is a very little development of secondary xylem in the shoots of *Bombax malabaricum* DC. and a very large development of secondary phloem occurs in it. The cambium activity terminates in December or January and is quiescent during the leafless phase. The production of flowers does not induce the cambium activity. It is difficult to conclude at this stage of investigation whether the inception of vegetative phase is caused by the resumption of meristematic activity or whether the vegetative growth induces the latter. But it is evident that both occur simultaneously. The phenomenon of leaf-fall can also be correlated with the activity of cambium. The fresh formation of conductive tissue is necessary for the continued vegetative growth and when the development of the secondary elements ceases the leaf-fall occurs. The termination of the vegetative growth is not abrupt but is gradual and it is also noticed that the termination of cambium activity is also gradual. In the case of the two species of *Cassia* the development of the secondary wood is normal and the cambium activity becomes very low at the termination of the vegetative growth. In the case of *Poinciana regia* Bojer. the cambium activity persists all throughout the year as there is no clear and definite termination of the vegetative phase in the tree as a whole but individual shoots may show

the termination of vegetative growth. Thus the vegetative phase is always accompanied by the cambium activity while during the reproductive phase the cambium is not active. It is also clear that during the vegetative phase the food materials are needed for the production of the new foliage as well as the production of the new elements of the conducting tissues while during the reproductive phase the food substances are needed for the production of flowers and fruits.

The results of the carbohydrate and nitrogen analyses are particularly interesting as the flowering phase and the vegetative growth show definite correlation with the carbohydrate contents and the C/N ratio. During the vegetative and reproductive phases the carbohydrate contents are higher than at any other period during the year. The nitrogen contents on the other hand show a continual rise from the beginning of the vegetative phase upto the end of the flowering period when there is a sharp decline in the total nitrogen contents. (In the case of *Bombax malabaricum* DC. there is a fall in the nitrogen contents at the end of the vegetative period.) There is some indication of second period of vegetative activity during the months of December and January in *Poinciana regia* Bojer. and in the month of January in the two species of *Cassia* and this second period of active vegetative growth is also marked by high carbohydrate contents. The high carbohydrate contents at the vegetative and reproductive phases can be attributed to the gradient of carbohydrate stream in opposite directions during the two phases. During the vegetative phase there must be active photosynthesis and a downward flow of carbohydrate occurs which may be responsible for the high carbohydrate contents of the shoots bearing leaves. The carbohydrates are probably stored up lower down and at the termination of the vegetative activity the shoots show a decline in the carbohydrate contents. When the flowering phase sets in there is an upward current of the carbohydrates and the gradient is established in the upward direction as they are needed for the formation of flowers and fruits. It is likely that the nitrogenous substances are needed in large amounts for the production of fruits than in the development of leaves and flowers and they, therefore, accumulate during the vegetative phase and are utilised at the end of reproductive phase.

These marked fluctuations in the carbohydrate contents during the two phases bring about marked differences in the C/N ratios. During the vegetative phase of the two species of *Cassia* the C/N ratios are highest while the C/N ratios have low values during the reproductive phase. In the case of *Bombax malabaricum* DC. and *Poinciana regia* Bojer., the C/N ratios have nearly the same values. *i.e.*, the vegetative and reproductive phases are not characterised by different C/N ratios. In all the four cases the C/N ratios are very low during the termination of vegetative and reproductive phases, *i.e.*, the inactive stages of the shoots are characterised by

low C/N ratios. It is very likely that if the flow of carbohydrate materials is interrupted or diverted to some other region of the shoot which would have normally flowered, it would not flower at all, as in the case of *Bombax malabaricum* DC. or would begin to grow vegetatively in the following season. In the case of two species of *Cassia* and in *Poinciana regia* Bojer., it would begin to grow vegetatively. During the active vegetative growth the values of C/N ratios rise but these rises are due to photosynthetic activity of the leaves. These higher values are a result and not the cause of the vegetative activity in one case and the reproductive activity in the other, as in the former case the photosynthetic activity is responsible for the higher C/N ratios and in the latter case the upward flow of the carbohydrate contents bring about high C/N ratios. As soon as the vegetative growth sets in, the cambium resumes its periodic activity and promotes increase of vegetative growth.

In nature the vegetative growth begins when the C/N ratio is lowest and it is accompanied by the resumption of the cambium activity. At the end of the vegetative activity the C/N ratio begins to fall on account of the translocation of the carbohydrates lower down. The inception of the reproductive phase occurs when the C/N ratio is low and is not accompanied by cambium activity.

Summary

Carbohydrate/nitrogen ratios of the vegetative and reproductive phases of (1) *Bombax malabaricum* D.C., (2) *Cassia renigera* Wall., (3) *Cassia fistula* Linn, and (4) *Poinciana regia* Bojer. are determined with a view to understand the internal causes governing the inception of the two phases.

The results show that the carbohydrate contents of the shoots (with leaves and flowers removed) are very high during the active vegetative and reproductive phases.

The total nitrogen is continually rising from June to April after which it shows a fall. The vegetative growth is marked by the highest values of C/N ratios in the two species of *Cassia* and in *Poinciana regia* Bojer. the C/N ratios are medium during the reproductive phase. In *Bombax malabaricum* DC. the C/N ratios are highest during the two active phases. The high values of C/N ratios are due to high values of carbohydrate contents. The high values of carbohydrate contents during the two phases are due to photosynthetic activity in the first case and to the upward flow of carbohydrates from places of storage in the second case. So these high C/N ratios are the effects rather than the causes of the vegetative and reproductive growth.

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OCCURRENCE OF EXTRACARPELLARY OVULES ON THE FLORAL AXIS IN COTTON

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Received for publication on 30th August, 1934.

Introduction

In the course of a general study of the anatomy of cotton flower an interesting case of production of extracarpellary ovules directly from the floral axis has been observed in a cross between *Gossypium herbaceum* Linn. (strain 2405) and *G. neglectum* (Cernuum). Such an occurrence of ovules on the floral axis has never been recorded before in cotton where ovules are normally produced on the carpels.

In cotton, central proliferations of the floral axis of a different nature have been observed by a few other investigators. Ramanatha Ayyar* has observed a supernumerary whorl of carpels inside bolls (Fig. 1) in large numbers in Bourbon (*G. purpurascens* Poir.) × Cambodia (*G. hirsutum* Linn.) crosses and in some apparently pure types of *G. hirsutum* L. at the Cotton Breeding Station, Coimbatore. Hubbard (6) has found on a single plant in a field of Acala cotton some peculiarly shaped bolls which when opened showed a well developed flower bud in the centre, surrounded by the inner margins of the carpels. The flower bud was axially placed, well developed, but without involucre bracts. He has also mentioned instances where supernumerary whorls of carpels were found within the bolls of the same variety.

Worsdell (9) mentions the occurrence of supernumerary whorls of carpels in Tulip, *Cerastium quarternellum* and the Naval orange. Bergman (2) has described the intracarpellary pistils and "double" flowers which he found as central proliferations of the floral axis in *Hibiscus*. He mentions that Harris has described similar proliferations in *Passiflora* and *Carica Papaya* and that Harris has observed intracarpellary fruits in capsules of *Hibiscus esculentus*. Bergman (2) cites also the case of capsules of *Hibiscus tiliaceus* L., where Delavaud has observed small capsules with aborted ovules within the ordinary capsules.

* Unpublished records referred to with permission.

Material and Method

The present case of abnormal production of naked ovules on the floral axis in cotton was observed while examining free hand sections of ovaries of the above mentioned crosses. Transverse sections of ovaries of a large number of F₁ plants were examined under the microscope with a view to study the anatomical peculiarities of flowers in hybrids. Flowers were also examined of a number of pure varieties of cotton.

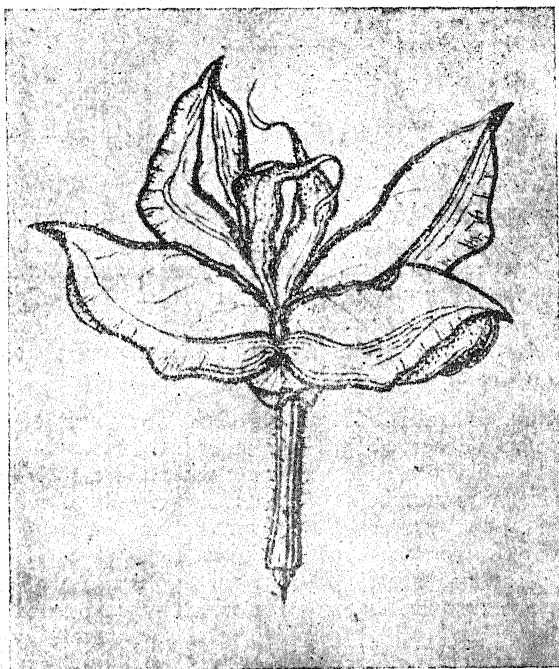


FIG. 1. Sketch of an open boll of a mixed stock of *G. hirsutum* Linn. (Cambodia) showing a whorl of supernumerary carpels. The additional whorl has two carpels. (After Ramanatha Ayyar). Natural size.

Observations

Among the specimens obtained from these hybrid plants of the first filial generation, in a number of flowers of different individual plants, the floral receptacle was observed to prolong upwards in the shape of a short rod, tapering towards the top, in between the united margins of the carpel, *i.e.*, in the centre of the placental axis of the ovary. In eight flowers from different plants this receptacular prolongation was found to carry at its tip a single bare ovule surrounded by the carpels (Plate I, A). The carpels were found to slightly expand around this ovule giving it sufficient room between

their united margins (Plate I, c). This ovule was fully developed and resembled in all respects the normal ovules borne at the margins of the carpels (Plate I, c). The section gives a clear idea of even the internal structure of the ovule (Plate I, c). The position of the ovule was such that it could not get fertilised as the pollentube-conducting tissue was not in direct communication with the space in which the ovule was located*. Not only was this ovule not borne on the margin of a carpel but appeared to represent the continuation or termination of the axis (Plate I, A). Transverse sections of the axis above the carpels clearly showed the receptacular vascular cylinder re-formed after the departure of the last carpellary traces (Plate I, B). Transverse sections at the place of attachment of the ovule on the axis revealed that into this solitary ovule passed all the residual vascular tissue of the axis, *i.e.*, the stele above the last traces to the carpels. It was this fact that made it appear to represent the termination of the axis.

In a few other instances it was observed that a single well developed ovule was produced on the bare surface of this prolongation, laterally, near the apex. When the ovule was borne laterally, that portion of the axis bearing this ovule showed certain structural changes. The receptacular stele had split up here into three distinct vascular strands (Plate I, D) to be reunited again into a single cord above the position of the lateral ovule. This splitting of the vascular cylinder was accompanied by a flattening of the axis and an attempt of this flattened portion to fold (Plate I, D) accommodating the ovule in the space between the folded halves of the axis. It is quite reasonable to suspect this to be an attempt to form a carpel with a median and two lateral traces and may be taken as an indication of the three-trace nature of the carpel. The probability of the primitive carpel being a three-trace organ has been emphasised by Eames (3) and other earlier workers. It should be noted that the solitary ovule in such cases was fed by one of the lateral strands as is the case in normal carpels. In one specimen it was noticed that the median of the three strands was very much smaller and less prominent than the lateral ones. This is certainly striking in view of the greater development of the marginal ovule-feeding traces of the normal carpels especially in cotton.

In the course of examination of several species of cotton, it has been observed that in many of them the receptacular stele extends beyond and above the place of origin of the carpellary traces. The extent to which the receptacular stele goes above the origin of the last carpellary traces, varies from species to species and even from

* Note.—Since this paper was sent to the Press a few plants from the progeny of X-rayed *G. indicum* (pure strain 546) grown at the Cotton Breeding Station, Coimbatore, produced bolls having a well developed seed, with lint, on the central axis. Fertilization was possible on account of the pollentube-conducting tissue being in direct communication with the central axis in such instances.

flower to flower within a species. In the strain C. 7, belonging to the species, *G. indicum* Gammie, it extends above the carpellary traces in almost all flowers, with a very few exceptions where it is represented at least by three or four vascular strands going upwards for a short distance and fading away. Another strain 546 of the same species, gives flowers showing the stele above the carpellary traces and flowers without it as such. Varieties like Roseum and Cernuum of the species *G. neglectum* have many flowers with the stele re-formed above the carpellary traces, others with only strands in its place and very few without even those strands. In many of the varieties belonging to the species *G. herbaceum* Linn., it rarely extends as such above the carpellary traces. In most of the flowers of this species no residual vascular tissue is left after the last carpellary traces have departed from the stele. In other words, into the carpels passes all the residual vascular tissue remaining after traces to the lower whorls have left. But in some flowers a few strands could be seen in its place.

With regard to this feature, Bourbon (*G. purpurascens* Poir.) and Cambodia (*G. hirsutum* Linn.) of the New World group of cottons show a condition similar to that obtained in Roseum and Cernuum of the Old World group, i.e., the receptacular stele is seen above the carpel traces in many flowers and in the others they are either represented by strands or as in rare cases entirely absent. The variations of this feature met with in the numerous species of the genus *Gossypium* is under study and it is hoped that it will be fully dealt with in a future communication.

As mentioned above, occasionally in the pure types which show the stele above the carpellary traces, but more frequently in some hybrids like *G. herbaceum* \times *G. neglectum* (Cernuum and Bourbon \times Cambodia), the floral axis is found to extend upwards, between the carpels, in the shape of a rod, with the receptacular stele extending into it. It was on this prolonged floral axis that the developments and proliferations described have been found to occur.

Discussion

Abnormal developments of this type are generally considered to be of great value in that, very often they throw considerable light on the ancestral history of the organ concerned or may give some clue to the solution of intricate problems.

Worsdell (9) is of opinion that the proliferations of the carpel whorl, in the cases which he cites, may be due to the "presence of a floral axis in the centre of the fruit and an extension upwards . . . of the floral axis".

Bergman (2) states that the intracarpellary pistils observed by him in *Hibiscus* "were in all cases, proliferations of the central axis of the capsule in which they were included".

The observations made in cotton fully bear out the same fact. Besides, the part played by the receptacular stele in the development and proliferations of the central floral axis above the level of the carpellary traces in cotton is clearly brought out.

The apices of various types of shoots of limited growth like that of the flower is morphologically and anatomically complex. Only a clear understanding of the behaviour of shoot tips in general will help to solve the many complexities of structure met with in the floral receptacle. Arber (1) emphasises the need for the study of apical peculiarities of shoots of limited growth, as she believes "that it may be one of the most hopeful directions in which to expect light on floral morphology". There has been much discussion in recent years on the nature and behaviour of the vascular cylinder at the apex of the floral receptacle. Saunders (7 and 8), in discussing the theory of carpel polymorphism, considers all such vascular tissue above the level of the last carpellary traces as of no consequence and merely "discarded" being "superfluous" tissue. The continuation of the vascular cylinder above the level of the last carpellary traces is a feature not only of many varieties of cotton but of several other plants. Eames (3) cites *Aquilegia* and *Prunus* as instances. He calls attention to the behaviour of the end of the floral vascular cylinder in some members of the Leguminosae and states that in "*Albizzia* the cylinder does not extend beyond the trace origins, though in very many genera, for example, *Bauhinia*, considerable arcs of the cylinder are dropped out at the level". Almost parallel conditions have been described above in the case of different species of cotton. Starting from *G. indicum* and going up to *G. herbaceum* the gradual disappearance of the stele above the trace origins, may be noticed. This clearly indicates that just as in the instances described by Eames (3), in varieties of cotton like *G. herbaceum* (H. 2405), the vestigial vascular cylinder tip above the trace origins has disappeared under greater specialisation, reduction and loss. In such cases the carpels may appear to represent the termination of the axis but actually only be pseudoterminal. Abnormal developments and proliferations in this portion of the floral receptacle can easily be understood "if the upper stelar vascular tissue is recognised for what it is" (3). It is not probable that "superfluous" vascular tissue "discarded into the pith"—as it may be considered by Saunders (7 and 8)—is involved in such developments which are characteristics of the floral axis.

The apparent terminal position of the solitary ovule at the apex of the axis and the passage of the whole of the residual vascular tissue into it, need not necessarily show that it is a terminal structure. It may only be lateral (pseudoterminal) representing perhaps an extremely reduced condition of a carpel. In this connection attention may be drawn to the new theory recently put forward by Hamshaw Thomas (5) with regard to the origin and development of ovules in Angiosperms. According to him "the ovules are held to represent original terminal structures, the placentae separate

branches and the carpel-wall a cupular structure which is quite distinct in origin from a typical foliar structure". It is difficult to say at the present stage, how this theory of terminal origin of ovules can be made to interpret the present phenomenon described above.

These kinds of proliferations are almost always met with only in plants of a hybrid nature and, as Hubbard (6) and Bergman (2) think, may be due to genetic disturbances resulting from combinations of different types. Bergman (2), agreeing with Goodspeed and Clausen (4), states that such "disturbance is probably due to inharmonious elements or factors in the gametes derived from plants of different species".

In this instance the abnormal production of ovules on the floral axis has been found in a number of individuals and that in the first filial generation of the cross. This leads to the suspicion that these developments are the result of intensification of features incompletely exhibited in the parents, because one of the parents of these hybrids producing such proliferations has the receptacular stele above the trace origins and sometimes even show prolongation of the axis beyond the normal floral whorls.

Summary

1. Production of extracarpellary ovules directly from the floral axis above the level of the carpels have been observed in a number of flowers of a cross between *G. herbaceum* Linn. and *G. neglectum* (Cernuum).

2. In several instances the floral axis was found to prolong upwards beyond the carpels and in some cases this axis was found to produce an ovule on it.

3. The part played by the receptacular stele in developments of this type is brought out and discussed.

4. The occurrence of such central proliferations is attributed to the prolongation of the floral axis and with it the receptacular vascular cylinder in one or both of the parents of the hybrids showing such proliferations.

5. Genetic disturbances resulting from hybridisation are suggested as the direct cause of such phenomena in hybrids.

ACKNOWLEDGMENT.

I am indebted to Mr. V. Ramanatha Ayyar, Cotton Specialist, Coimbatore, for his very helpful guidance and suggestions during the course of this work.

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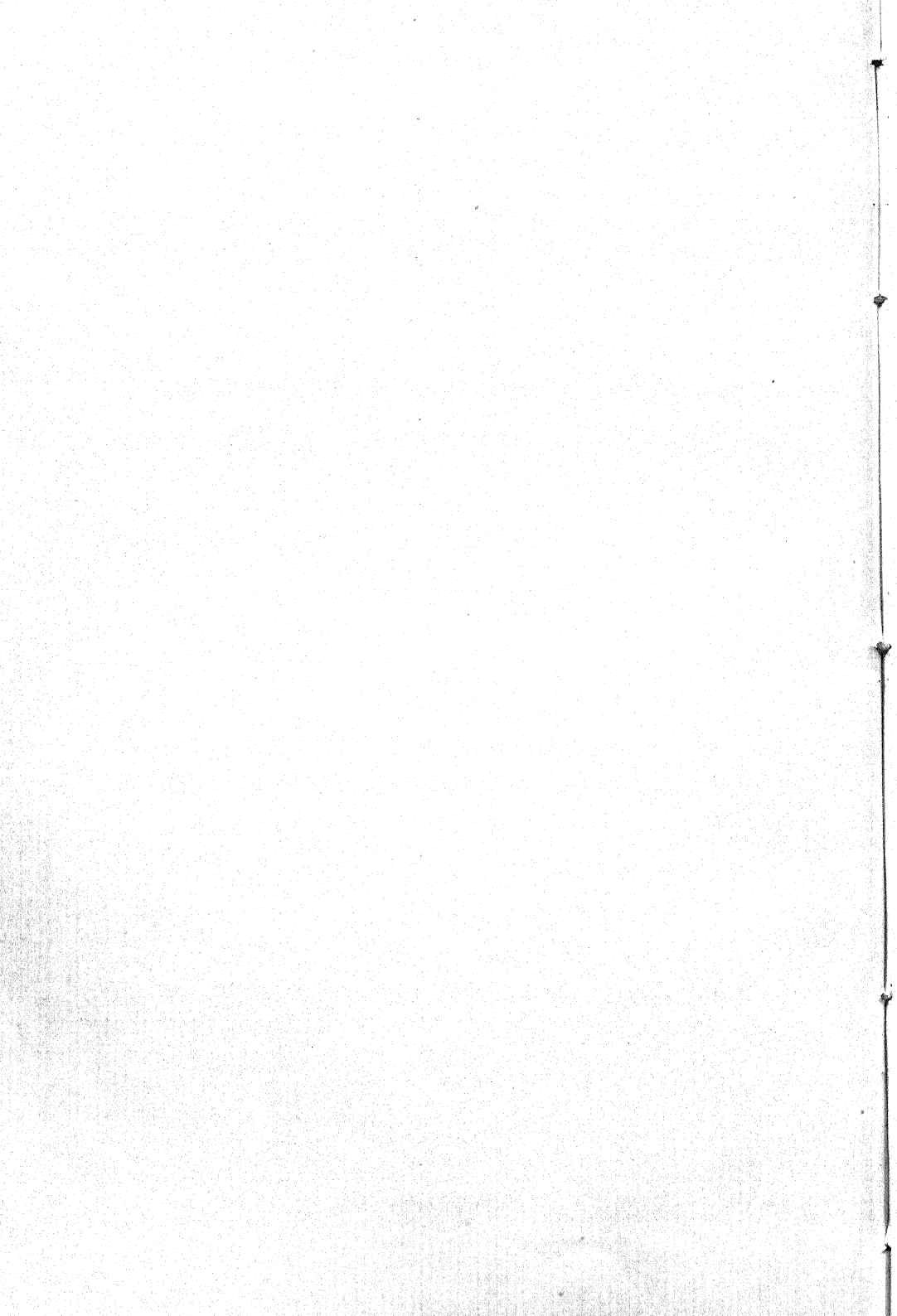
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Explanation of Plate I

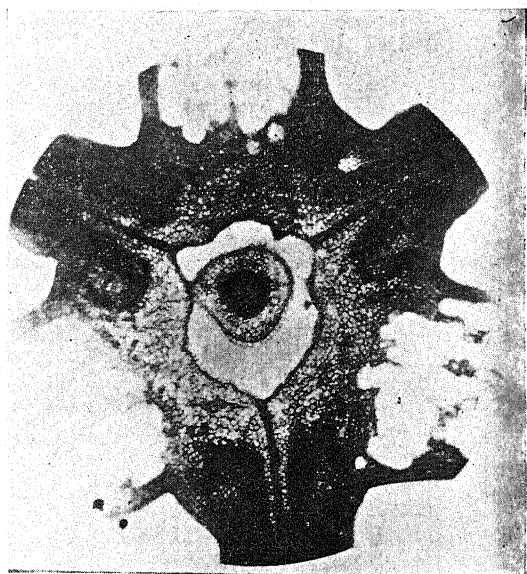
The figures given in the plate are all photomicrographs.

- A. The solitary naked ovule at the tip of the floral axis, photographed after removing the carpels. (*G. herbaceum*) \times *G. neglectum* (Cernuum) F₁. \times 10.
- B. Transverse section showing the floral axis in the space between the united margins of the carpels. Note the receptacular stele in the axis. \times 80.
- C. Transverse section passing through the ovule. Note the internal structure of the ovule. The ovule is surrounded by the carpels. \times 80.
- D. Transverse section showing the floral axis flattened, slightly folded, with the stele broken up into three distinct strands. \times 80.

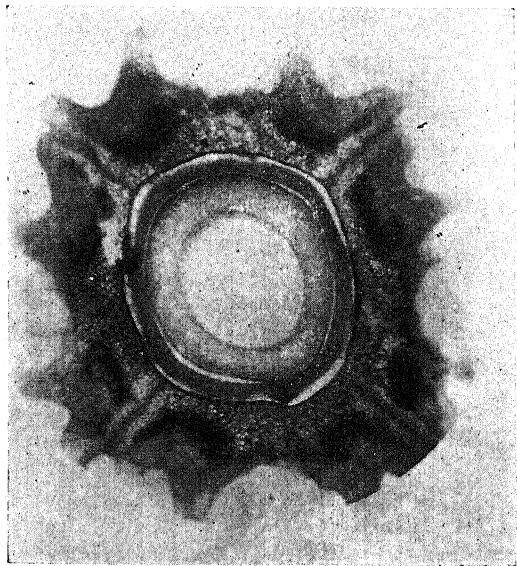




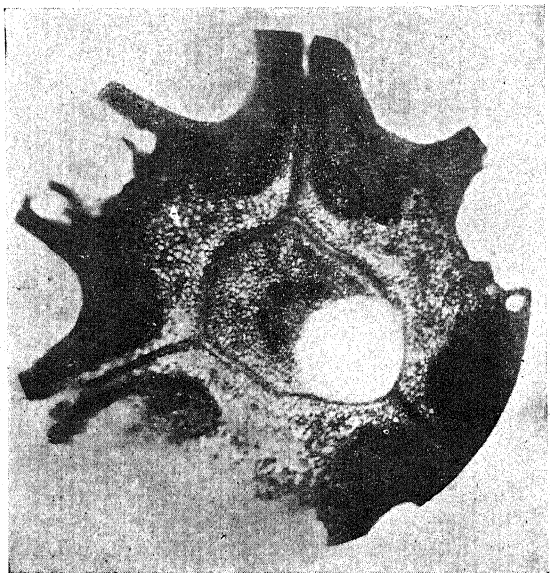
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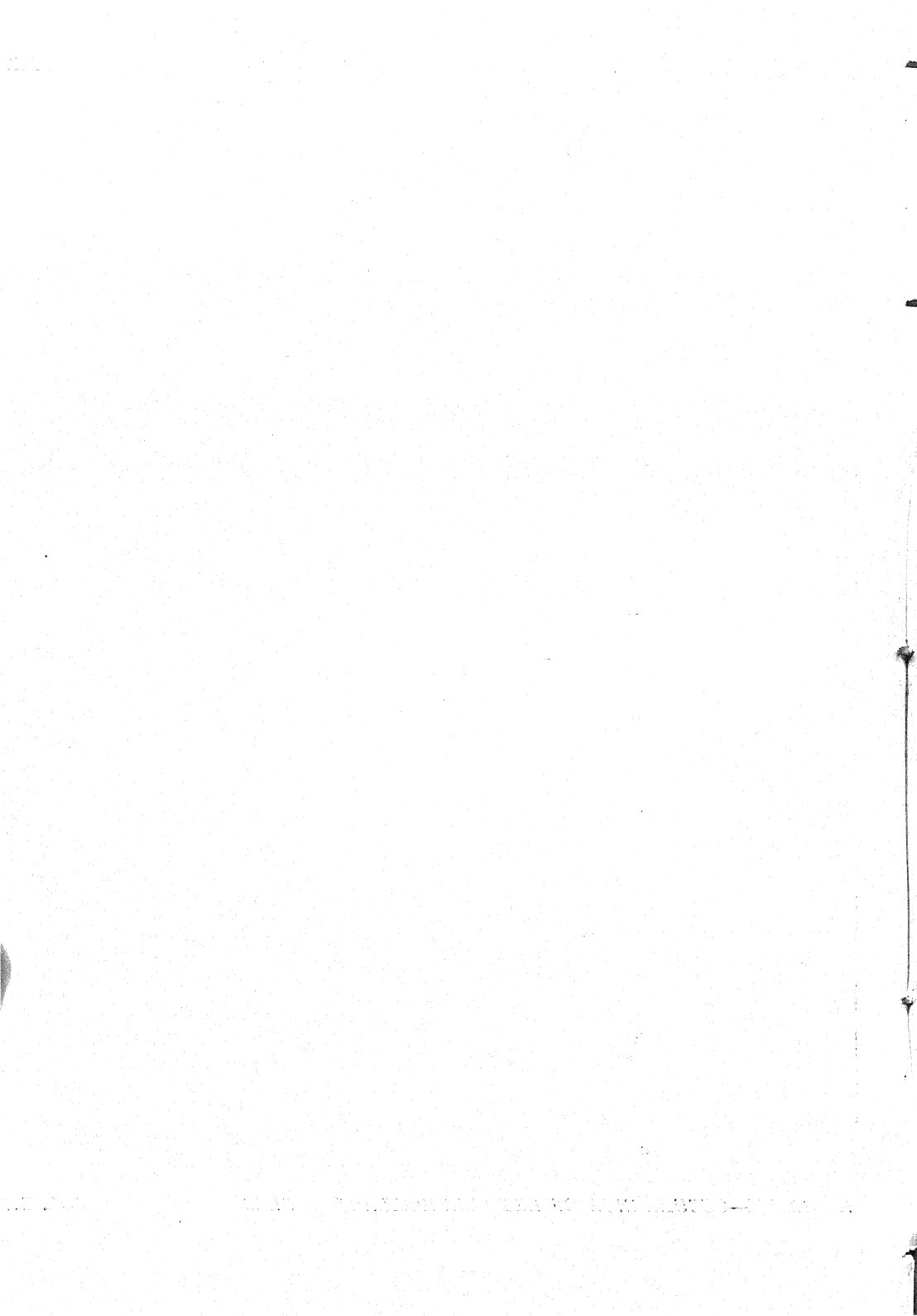
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D



THE EFFECT OF WOUNDING ON RESPIRATION IN THE STARVING LEAVES OF *ARALIA* *GUILFUYLEI**

BY

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Received for publication on 10th August, 1934.

Introduction

The effect of wounding on respiration has been studied by various investigators, chief amongst whom are Palladin (5), Boehm (1), Lutman (3), Magness (4), Stich (7), Johnstone (2) and Richard (6). In almost all such cases studied it has been demonstrated that wounding increases the respiratory activity of the organ concerned. Feeding uninjured plant organ with sugar solution of low concentration also increases its carbon dioxide output (5). But as far as the author is aware, there is no reference to any work with regard to the amount of available respirable material present in a plant organ at the time of wounding and its relation to the enhanced respiratory activity after the operation. A thorough study of the problem indicated has been made on the leaves of *Aralia Guilfuylei* and the results are presented in the following paragraphs.

Material and Methods

A--Preparing leaves for the experiment.

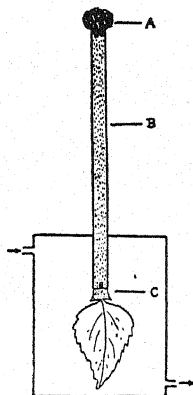
(i) Selection of the leaf. In all cases, the leaves were isolated at 9-30 A.M. from the same plant and approximately from the same side, sufficient care being taken to select leaves of the same age.

(ii) Method of starving the leaf. Soon after removal, the leaves were accurately weighed and kept, with their petioles dipping in water, in a dark chamber through which a continuous current of air was drawn. After a definite period of starvation, at which the effect of wounding was to be studied, the leaves in question were removed from this chamber to another, where the respiration was measured. Both these chambers were kept at a constant temperature of 26°C.

(iii) Injection of glucose solution into the leaf. After 24 hours of starvation, the leaves were injected with 1.5% glucose solution under pressure. The method consisted in making the

*This problem was undertaken and completed in the Botanical Laboratory, Ravenshaw College, Cuttack.

petiole of the leaf secure in a rubber bung through a suitable hole. The bung was then tightly inserted in a long (30 inches) thick glass tube of wide bore, which was held in a vertical position with the starved leaf at its lower end hanging freely in a chamber, maintained at 26°C, (Text-fig. 1) through which a slow current of air was drawn. The glass-tube, so arranged, was filled with 1.5% glucose solution and the apparatus was allowed to run for 24 hours. A little of toluol was added to the sugar solution at the open end of the glass tube to prevent bacterial growth and it was finally plugged with cotton-wool.



Text-fig. 1. Arrangement of the apparatus showing the method by which sugar-solution was injected into the leaf. A = Cotton wool to plug the open end of the glass tube. B = Glass tube filled with sugar-solution. C = Rubber bung in which the petiole of the leaf was inserted.

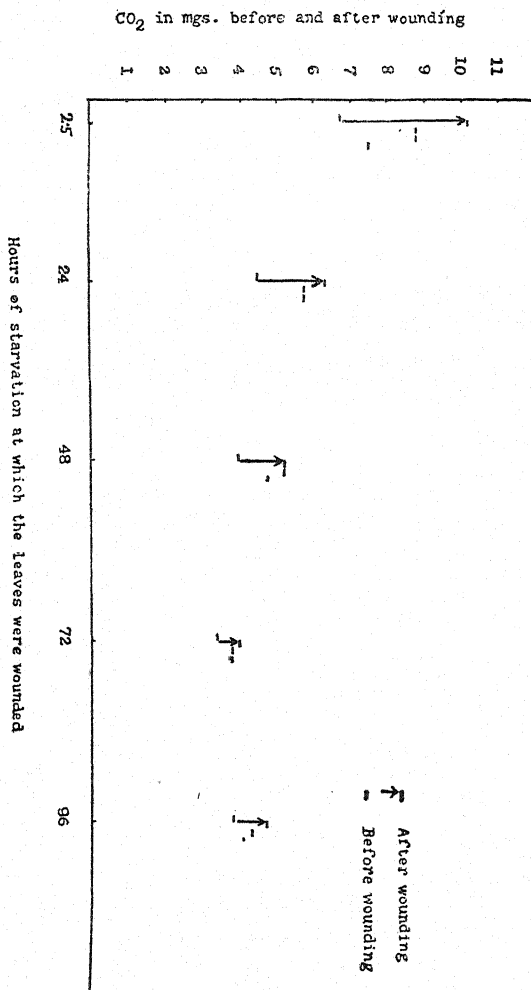
To gain some idea, by way of check, regarding the efficiency of the method employed, the cell-concentrations of the sugar-injected and control leaves were measured by plasmolytic method with KNO_3 as the plasmolysing medium. An increase by about 6.63 to 8.59 atmospheres was noticed in the sugar-fed leaves as compared to the control.

(iv) The wounding of the leaf. The leaves were wounded between the veins by making fine incisions with the help of a sharp scalpel. The same amount of injury was done in all the cases studied. The injured portion was soon after, wiped with a moist cloth.

B—*Estimation of carbon dioxide.*

The respiration of the experimental as well as of the control leaves was measured simultaneously at a constant temperature of 26°C. The two-hourly march of respiratory output of CO_2 was measured in each case before and after wounding. The estimation of CO_2 was done by the continuous current method under identical

conditions. The respiratory value was measured by the rate of CO_2 produced per 10 grms. fresh weight of the leaves.



GRAPH I showing the rise in the respiratory activity after wounding at various stages of starvation.

The experimental leaves (not fed with sugar) were wounded at six different periods of starvation, namely $2\frac{1}{2}$, 24, 48, 72 and 96 hours, while the sugar-fed leaves were wounded at 48 hours of starvation.

Results

By reference to Graph I it will be seen that the effect of wounding, just after $2\frac{1}{2}$ hours of starvation, is maximum. This is

TABLE I
Showing the nature of the after-effect of wounding the sugar-fed and the control leaves at 48 hours of starvation.

48 hours starved wounded leaves.		Initial output of CO ₂ in mgs. before wounding.	The after-effect. The difference between the initial output of CO ₂ before and after wounding.	The percentage increase of the after-effect.	The percentage increase of the after-effect over that of the control.
Sugar-fed	..	6.4	2.9	45.3	8.8
Control	..	4.1	1.5	36.5	..
Sugar-fed	..	6.6	3.0	45.4	10.4
Control	..	4.0	1.4	35.0	..
Sugar-fed	..	7.3	3.6	49.3	12.1
Control	..	4.3	1.5	37.2	..
Sugar-fed	..	8.6	4.9	53.4	18.4
Control	..	4.0	1.4	35.0	..
Sugar-fed	..	9.4	5.6	59.1	25.1
Control	..	4.4	1.5	34.0	..
Sugar-fed	..	12.4	0.0	0.0	..
Control	..	4.5	1.7	37.7	..
Sugar-fed	..	12.8	— .9	— 7.0	..
Control	..	3.9	1.4	35.8	..
Sugar-fed	..	14.0	— 2.8	— 20.0	..
Control	..	4.2	1.5	35.7	..

expressed in terms of the high value of the respiratory output of CO_2 , which is immediately followed either by a jerky depression, or by one (rarely two) level value, which again is succeeded by falling values. A similar rise in the after-effect was noticed in leaves starved for 24, 48 and 72 hours except for the intensity of the after-effect, which shows falling values as the period of starvation increases; while on the contrary at 96 hours of starvation, there is an un-expected rise in the value of the after-effect as compared to the 72 hour starved leaves.

Higher respiratory activity is indicated in all the sugar-fed leaves of *Aralia Guilfuylei*; and in the same, on wounding it has been noticed that the respiratory activity is still further enhanced. Of the latter phenomenon the study has been made up to a maximum of 48 hours. The difference between the values of the after-effects of wounding for injected and control leaves at the same period of starvation varies usually from 1.4 to 4.1 mgs. Expressing these after-effects in percentage and calculating the mean thereof, it has been found that the average increase in the after-effect for the sugar-fed leaves over that of the control is 14.9% (Table I). The maximum after-effect was, however, perceived in those sugar-fed leaves which had initial respiration values at 9.4 mgs. (per two hours). While on the other hand the sugar-fed leaves having higher initial values (12.8 mgs. onwards) did not display any rise but indicated a jerky fall immediately following the operation except in one case, where initial value was at 12.4 mgs. level (per two hours). Curiously enough the jerky fall came about after 2 hours.

Discussion of Results

The gradual fall in the intensity of the after-effect as the period of starvation increases, is most likely due to a combined effect of a gradual inactivation of the protoplasm and decrease in the respirable material.

The unexpected rise in the after-effect values in leaves starved for 96 hours may be due to a change in the permeability of the cells, leading to the accessibility of more of respirable material. This possibility is clear from the control experiment where there is a rise in the output of CO_2 at the same stage of starvation. The rise in the values of the after-effect of the sugar injected leaves, over that of the control, may likewise be attributed to a similar increase (over that of the control) in the respirable material supplied artificially.

It is my pleasant duty to express my great indebtedness to Prof. P. Parija, M.A. (Cantab), B.Sc., (Cal.), I.E.S., for his unflinching help, critical suggestions and able guidance throughout the course of this investigation besides offering me enormous facilities in his laboratory.

Summary

The effect of wounding on the respiratory activity of the starving leaves of *Aralia* and on such leaves which were injected with 1.5% glucose solution, was studied with a view to ascertain whether the respirable material present in the leaves at the time of wounding has any relation to the enhanced CO₂ output following wounding. The results obtained go to show that there is—

(1) a gradual fall in the intensity of the after-effect of wounding, as the period of starvation increases from 2.5 hours to 72 hours,

(2) an unexpected rise in the after-effect at 96 hours of starvation,

(3) a rise in the values of the after-effect of the sugar-injected leaves over that of the control and

(4) a lack of any after-effect in the sugar-injected leaf where higher initial values for CO₂ output were involved.

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NOTES ON A COLLECTION OF PLANTS FROM MAHENDRAGIRI

BY

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Received for publication on 14th May, 1935.

In an excursion conducted by Professor P. Parija to the Mahendragiri Hills in Ganjam during the Christmas holidays of 1934, the following plants were collected. This note was prepared with the idea of finding out any indication as to how far sub-tropical plants are represented in the flora of a high hill lying within the Tropics.

Mahendragiri hills are a part of the chain of Eastern Ghats passing through the Ganjam District and is situated in the middle of it at an approximate distance of 15 miles from the Bay of Bengal and lies at about 19°-19' N. and 84°-20' E. with an altitude close upon 5,000 feet.

The plants collected are classified below:—

Name of family.	Name of plant.	Habitat.	Other localities where occurring.
1	2	3	4
Acanthaceæ	1. <i>Hemigraphis latebrosa</i> Nees.	3,-4,000 ft. (shade)	Deccan—0-4,000ft. South Behar.
	2. <i>Echinacanthus attenuatus</i> Nees.	3,-4,900 ft.	C. and E. Himalaya 2,-4,000 ft.
	3. <i>Thunbergia tomentosa</i> Wall. or T. <i>Fragrans</i> var. <i>vestita</i> Nees.	1,-2,000 ft. (shade). ..	South Deccan and Ceylon.
	4. * <i>Strobilanthus Jey-porensis</i> Bedd.	4,900 ft.	Hills of Golkonda 2,500 ft. and Jeypur.
	5. <i>Justicia simplex</i> Don	At all heights.	Hills of W. Peninsula upto 4,000 ft.

Name of family. 1	Name of plant. 2	Habitat. 3	Other localities where occurring. 4
Asclepiada- ceæ.	6. <i>Gymnema sylvestre</i> Br.	Foot of hill.	
Balanophoro- raceæ.	7. * <i>Balanophora polyandra</i> Griff.	4,000 ft. a root parasite	Sikkim Himalaya on roots of trees, alt. 4,-6,000 ft. Khasia Mts.
Campanula- ceæ.	8. <i>Campanula canescens</i> Wall.	4,000 ft. and above (shade)	Throughout N. India 0,-5,000 ft. in Himalaya
Capparida- ceæ.	9. <i>Capparis grandis</i> L.	Foot of hill.	
Celastraceæ	10. <i>Gymnosporia emarginata</i> Roth	1,-4,000 ft.	Concan, W. Peninsula, Ceylon, Himalaya.
Compositæ	11. <i>Conyza stricta</i> Willd.	1,-2,000 ft.	Sub-trop. Himalaya upto 5,000 ft. and Khasia.
	12. <i>C. japonica</i> Lees ..	4,000 ft.	Tropical Himalaya upto 5,000 ft. Khasia.
	13. * <i>Senecio corymbosus</i> Wall.	4,900 ft.	C. Provinces 7,000 ft. Nilgiri Hills.
	14. †† <i>S. nudicaulis</i> Ham.	4,900 ft.	Temp. Himalaya 5,-10,000 ft.
	15. <i>Vicoa auriculata</i> Cass.	1,-2,000 ft. (shade)	Trop. Himalaya upto 4,000 ft.
	16. <i>Laggera pterodonta</i> Benth.	2,000 ft. (shade)	Trop. Himalaya upto 4,000 ft.
	17. <i>Siegesbeckia orientalis</i> L.	1,000 ft.	
	18. * <i>Vernonia divergens</i> Benth.	1,-4,900 ft.	Paresnath Hills 4,000 ft. C. India, Concan.
	19. <i>Gnaphalium luteoalbum</i> L. var. <i>pallidum</i>	3,-4,900 ft.	Very common throughout India.
	20. <i>Gnaphalium luteoalbum</i> L. var. <i>multi-ceps</i> .	4,000 ft.	Orissa, Chota Nagpur, Himalaya, Khasia near the foot.

Name of family. 1	Name of plant. 2	Habitat. 3	Other localities where occurring. 4
Cucurbitaceæ.	21. <i>Bryonia scabrella</i> L.	4,000 ft.	
Cyperaceæ.	22. ** <i>Carex speciosa</i> Kunth.	3,-4,900 ft. crevices of rocks	Nepal to Sikkim and Khasia 1,-7,000 ft.
Euphorbiaceæ.	23. <i>Euphorbia perbracteata</i> Gage.	2,000 ft.	
	24. <i>Gelonium lanceolatum</i> Willd.	Foot of hill.	
	25. <i>Bridelia montana</i> Hook.	Foot of hill.	
Gentianeaceæ.	26. * <i>Swertia affinis</i> Clarke.	4,900 ft.	Deccan 2,-4,000 ft. and Chota Nagpur to Purneys.
	27. <i>Canscora diffusa</i> R. Br.	All over the hill side.	Throughout India ascending to 4,000 ft. Behar, Deccan, etc.
	28. <i>C. decussata</i> Roem. and Schult.	4,000 ft.	Throughout India ascending to 4,000 ft. Himalaya to Burma, etc.
Labiatae ..	29. * <i>Plectranthus menthoides</i> Benth.	3,-4,800 ft.	C. Provinces 4,-6,000 ft. and S. Deccan mountains.
	30. † <i>Scutellaria discolor</i> Colebr.	3,-4,000 ft. (shade)	Sub-Trop. Himalaya 1,-6,000 ft. Khasia Concan.
	31. * <i>Leucas montana</i> Spreng.	4,900 ft.	Behar, Deccan, Paresnath upto 4,500 ft.
	32. †† <i>Pogostemon plectranthoides</i> Desf.	500-3,000 ft.	Orissa Mahabinayak hills, W. Himalaya 1,-5,000 ft.
	33. <i>Colebrookia oppositifolia</i> Sm.	1,-2,000 ft.	Sub-trop. Himalaya 1,-4,000 ft. Behar, Deccan.
	34. <i>Nepeta ruderalis</i> Ham.	Foot of hill.	
Linaceæ ..	35. ** <i>Reinwardtia trigyna</i> Planch.	On top 4,900 ft.	Paresnath, all hilly parts of India.

Name of family. 1	Name of plant. 2	Habitat. 3	Other localities where occurring. 4
Loranthaceæ.	36. <i>Viscum articulatum</i> Burm.	At foot of hill.	Also collected from Salsette.
Lythraceæ..	37. <i>Woodfordia floribunda</i> Salis.	500 ft.	
Menispermaceæ.	38. <i>Cocculus villosus</i> Dc.	Foot of hill.	
Myrsinaceæ.	39. <i>Ardisia humilis</i> Vahl.	4,500 ft. (shade)	Throughout India 0,-5,000 ft.
Papilionaceæ.	40. <i>Crotalaria albida</i> Heyne.	All over the hill side.	Paresnath hills, in the plains, ascending to 7,000 ft. in W. Himalaya.
	41. ** <i>Indigofera pulchella</i> Roxb.	3,-4,000 ft.	Paresnath, throughout Himalaya upto 5,000ft.
	42. † <i>Shuteria vestita</i> W. & A.	2,-4,000 ft.	E. Himalaya, Khasia, hills of W. Peninsula and Ceylon.
Piperaceæ..	43. ** <i>Peperomia reflexa</i> A. Dietr.	Near top 4,000 ft. An epiphyte.	
Polygonaceæ.	44. ** <i>Polygonum chinense</i> L. var. <i>ovatifolia</i> .	3,-4,000 ft. (shade)	Himalaya, Paresnath Deccan, Ceylon.
Ranunculaceæ.	45. <i>Thalictrum foliolosum</i> Dc.	4,-4,900 ft.	Temp. Himalaya and Khasia.
	46. * <i>Clematis Wightiana</i> Wall.	3,-4,900 ft. (shade)	Hills of Deccan, Orissa, Concan; Nilgiri.
	47. †† <i>Clematis nutans</i> Royle.	3,000 ft. (shade)	Paresnath Khasia and W. Sub-trop. Himalaya.
Rubiaceæ ..	48. ** <i>Rubia cordifolia</i> Linn.	3,-4,900 ft.	All hilly districts up to 8,000 ft.
	49. ** <i>Hamiltonia suaveolens</i> Roxb.	2,-4,000 ft.	Paresnath hills, Himalaya Salt Range, hills of W. Peninsula 4,000 ft.
Rutaceæ ..	50. <i>Toddalia aculeata</i> Pers.	3,000 ft. (partial shade).	

Name of family.	Name of plant.	Habitat.	Other localities where occurring.
1	2	3	4
Rutaceæ ..	51. <i>Glycosmis pentaphylla</i> Corr.	Foot of hill.	Trop. Himalaya, Khasia.
	52. <i>Xanthoxylum budrunga</i> Wall.	3,000 ft.	
Santalaceæ.	53. <i>Santalum album</i> L.	Foot of hill.	
Scrophulariaceæ.	54. <i>Lindenbergia urticæfolia</i> Lehm.	1,000 ft.	
	55. ** <i>Sopubia trifida</i> Ham.	3,-4,900 ft.	Temp. & Sub-trop. Himalaya 3,-7,000 ft. Khasia.
	56. † <i>Alectra indica</i> Benth.	4,000 ft.	Temp. Himalaya 3,-9,000 and Khasia 8,000 ft.
Umbelliferæ.	57. <i>Pimpinella bracteata</i> Haines.	3,-4,900 ft.	Highest hills of Chota Nagpur 3,000 ft.
	58. * <i>Bupleurum mucronatum</i> W. & A.	4,000 ft.	Mountains of S. Deccan Ceylon 5,-8,000 ft.
Violaceæ ..	59. ** <i>Viola Patrinii</i> Dc.	4,900 ft.	Temp. Himalaya alt. 4,-8,000 from Kashmir to Bhotan, Khasia, hills of W. Peninsula and Ceylon.
Araliaceæ.	60. <i>Heptapleurum venulosum</i> Seem. var. <i>Roxburghii</i> .	Near top of hill.	Throughout tropical and sub-tropical India from N. W. Himalaya to South Deccan.

I. A glance over the foregoing collection of 60 specimens shows that they belong to 28 different families of which 17 families have one species each, the rest are represented by more than one. Compositæ with its 10 species preponderates over other families; next comes Labiatae with six species and Acanthaceæ with its five; after these come Euphorbiaceæ Gentianaceæ, Papilionaceæ, Ranunculaceæ, Rutaceæ and Scrophulariaceæ, each with three species. Last of all come Umbelliferæ and Rubiaceæ with two species each.

II. Of the 60 specimens 29, i.e., almost half the number, are plants of the hilly tracts with the probable exception of *Euphorbia perbracteata* Gage which is mentioned by Haines (3) as collected

from cultivated fields of Bihar and is considered by him to be an introduction. This plant has been collected from Mahendragiri hills at an alt. of 2,000 ft. where the climate seems to approximate that of the plains of Bihar. If these 29 plants are analysed and grouped according to the localities where they are commonly seen, it is observed that 5 of them may be considered Eastern Himalayan and 4 Western Himalayan. 10 of them are found in the Mountains of the Southern Peninsula and 9 are seen in all the hilly tracts of India.

One plant, *vis.*, *Pimpinella bracteata* Haines has been collected only from the highest hills of Chota Nagpur at an alt. of 2,500-3,000 ft. apparently by Haines (3) himself or his collectors. 3 of the specimens are found over all the Himalayas and also in Khasia of which one, *vis.*, *Thalictrum foliolosum* D.C. is found only in the temperate Himalaya 5,800 ft. and Khasia 4,600 ft., the other two at low elevations. Thus it will be noticed that in the sub-tropical plants collected, there is a preponderance of the South Indian hill flora; then come plants which are found in all hilly parts of India, after that the Eastern Himalayan and then the Western Himalayan species. In the above list, plants found on the mountains of the Southern Peninsula are marked*, those found in all hilly tracts are marked**, those of the Eastern Himalaya are marked† and those of the Western Himalaya are marked††.

III. Of the 10 species under Compositæ 4 are found in cold places mostly on the hills with a sub-tropical climate, *vis.*, Nos. 12, 13, 14 and 18; two others, *vis.*, Nos. 19 and 20 are found growing in the plains but in this case they were collected from an altitude of more than 3,000 ft., hence vary somewhat from the specimens of the plains as regards their colour of bract leaves, woolliness and height. They are more fully clothed with cottony tomentum, the colour of their involucre bracts are more golden and their height a little taller than usual, probably due to growing in shade. Of the specimens of Labiatae Nos. 29 and 30 are plants of sub-tropical regions whereas Nos. 31, 32 and 33 are found on the plains at higher latitudes such as North Bihar, but is seen at its best on hill sides upto an altitude of 4-5,000 ft. Amongst the Acanthaceae plants two may be considered sub-tropical, *vis.*, Nos. 2 and 4, the latter having rather restricted distribution; and No. 1 is found on the plains in Bihar but in Deccan Plateau sometimes rises upto 4,000 ft. One of the Gentianaceae, *vis.*, *Swertia affinis* Clarke is a hill plant preferring a cold habitat; 2 of the 3 Papilionates may be considered sub-tropical and are scarcely seen on the plains of Bihar or Orissa. The 3 specimens of Ranunculaceae are all sub-tropical plants and never seen in the plains or even on the low hills. Again 2 of the 3 Scrophulariaceae, *vis.*, Nos. 55 and 56 are found in sub-tropical, even in temperate regions on the Himalayas or Khasia. Both of the Umbelliferae Nos. 57 and 58 are sub-tropical plants and never

noticed on the plains. Of the two Rubiaceæ collected, No. 48 is decidedly sub-tropical.

Summary

In a survey of this list two points attract attention:

1. That among the sub-tropical plants collected from Mahendragiri hills there is a preponderance of the South Indian hill flora over other hill floras.

2. That in some cases slight changes in colour of floral leaves, in woolliness or other characteristics are brought on plants by their growing on hill slopes as is seen in the case of the 2 *Gnaphaliums*.

In concluding my remarks on the plants collected from the Mahendragiri hills, I wish to acknowledge with pleasure the kind help and guidance I received from Professor P. Parija, the Head of the Botany Department of Ravenshaw College.

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NOTES ON THE TERATOLOGY OF CERTAIN INDIAN PLANTS — VIII

BY

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Received for publication on 15th January, 1934.

The specimens described here comprise seventeen species spread over fifteen representative families. They are noted below with their respective localities.

Family.	Name of Plant.	Locality.
Piperaceæ ..	<i>Piper Betle</i> L. ..	Chainpore (Bihar).
Rosaceæ ..	<i>Rosa damascena</i> Mill. ..	Nainital.
Leguminosæ ..	<i>Dalbergia Sissoo</i> Roxb. ..	Indore.
Anacardiaceæ ..	<i>Mangifera indica</i> L. ..	Indore and Cuttack.
Balsaminaceæ ..	<i>Impatiens Balsamina</i> L. ..	Gohaun (Bihar).
Elæocarpaceæ ..	<i>Elæocarpus ganitrus</i> Roxb. ..	Barh (Bihar) and Deo Guraria (C. I.).
Vitaceæ ..	<i>Vitis vinifera</i> L. ..	Simla.
Malvaceæ ..	<i>Hibiscus Rosa-sinensis</i> L., <i>Gossypium neglectum</i> and <i>G. indicum</i> var. <i>malvensis</i> ..	Indore.
Combretaceæ ..	<i>Quisqualis indica</i> L. ..	Jehanabad (Bihar).
Myrtaceæ ..	<i>Psidium Guyava</i> L. ..	Gohuan (Bihar).
Convolvulaceæ ..	<i>Evolvulus alsinoides</i> Wall. ..	Benares and Cuttack.
Solanaceæ ..	<i>Lycopersicum esculentum</i> Mill.	Gohuan (Bihar).
Acanthaceæ ..	<i>Eranthemum atropurpureum</i> Hort. ..	Cuttack.
Rubiaceæ ..	<i>Morinda tinctoria</i> Roxb. ..	Ramnagar (U. P.).
Compositæ ..	<i>Tagetes erecta</i> L. ..	Gohaun (Bihar) and Sarnath (U. P.).

***Piper Betle* L.**

The abnormal leaf of *Piper Betle* L.* (*pán*) perhaps approximates to the condition seen in *Piper caninum* R. P. (with copious doubling of the leaves on the sides) and *P. porphyphyllum* N. E. Br. (with leaf-fork) (5).

The anomalous leaf described here consists of two tips** vividly recalling the two-tipped leaf of *Ficus religiosa* L. (9). The petiole is common (Fig. 16) but the mid-rib just a little higher up, bifurcates into two strands A and B. One of these (A) soon gives rise to three branches *a, b, c*. The branch *A* goes to supply the left fork of the tip; while the right fork is supplied by the original strand B.

Although this abnormal leaf apparently looks like a homologue of two normal leaves, a study of its size and venation is against such a conclusion.

***Rosa damascena* Mill.**

The phenomenon of phyllody of petals and petaloidy of sepals is very common indeed amongst the roses and fasciation of vegetative branches was observed by Geisenheyer (4); but the occurrence of triplet flower is very rare and henceforth unrecorded. This was observed at Nainital in October 1931.

In the specimen (Fig. 5) three flower buds are very easily distinguishable. Of these, two are included inside a common set of sepals while the lateral third is seated inside its own independent calyx. The pedicels of both are wholly fused up into a flat channelled structure. A section through this suggests a homologue of two pedicels. Its periphery is protected by a thick belt of stereome (Fig. 26) and the rest of the ground-tissue is thin-walled. The ring of stele is constricted opposite the grooves and looks like an hour glass. Outside this ring appear accessory vascular bundles in which like the primary bundles abundant secondary growth is seen (Fig. 27).

***Dalbergia Sissoo* Roxb.**

The abnormal flower observed is characterised by the presence of ten stamens as against the normal nine. This tenth stamen (normally abortive) peculiarly enough is a petaloid hooded structure with triangular thickened processes on either side (Fig. 14). The filament of this abnormal stamen towards the lower end is, however, confluent with that of a smaller one.

* Obtained through the courtesy of a friend in the year 1929.

** More recently Mr. K. L. Saksena (*Proc. 19th Indian Sc. Congress*, p. 331) of Gwalior, has recorded an abnormal leaf of *Piper Betle* with three tips.

***Mangifera indica* L.**

Here the abnormality consists in the very great variation of (Figs. 6-13) floral parts (see table below). A large number of flowers were examined and it has been found that flowers having $K_5 C_5$ are the commonest and next in order are those with $K_4 C_4$ and $K_5 C_6$. The other variations are rather rare. Flowers with K_6 or $K_8 C_9$ or C_{10} are bigger in size although the gynaecium is normal in each case. The order of alternation of petals with regard to sepals is as follows:—

Sepals and Petals.		Arrangement of petals with regard to sepals.	
		Sepals.	Petals.
$K_4 C_4$..	1-1-1-1	1-1-1-1
$K_5 C_5$..	1-1-1-1-1	1-1-1-1-1
$K_4 C_7$..	1-1-1-1	2-2-2-1
$K_4 C_5$..	1-1-1-1	1-1-1-2
$K_3 C_6$..	1-1-1	3-2-1
$K_3 C_9$..	1-1-1	2-4-3
$K_3 C_{10}$..	1-1-1	2-5-3

It appears that the lower number of sepals is due to the suppression of certain of the members, while on the other hand the higher number seems to be a result of a complete chorisis of petals. In one case with $K_5 C_6$ a petaloid stamen was noticed.

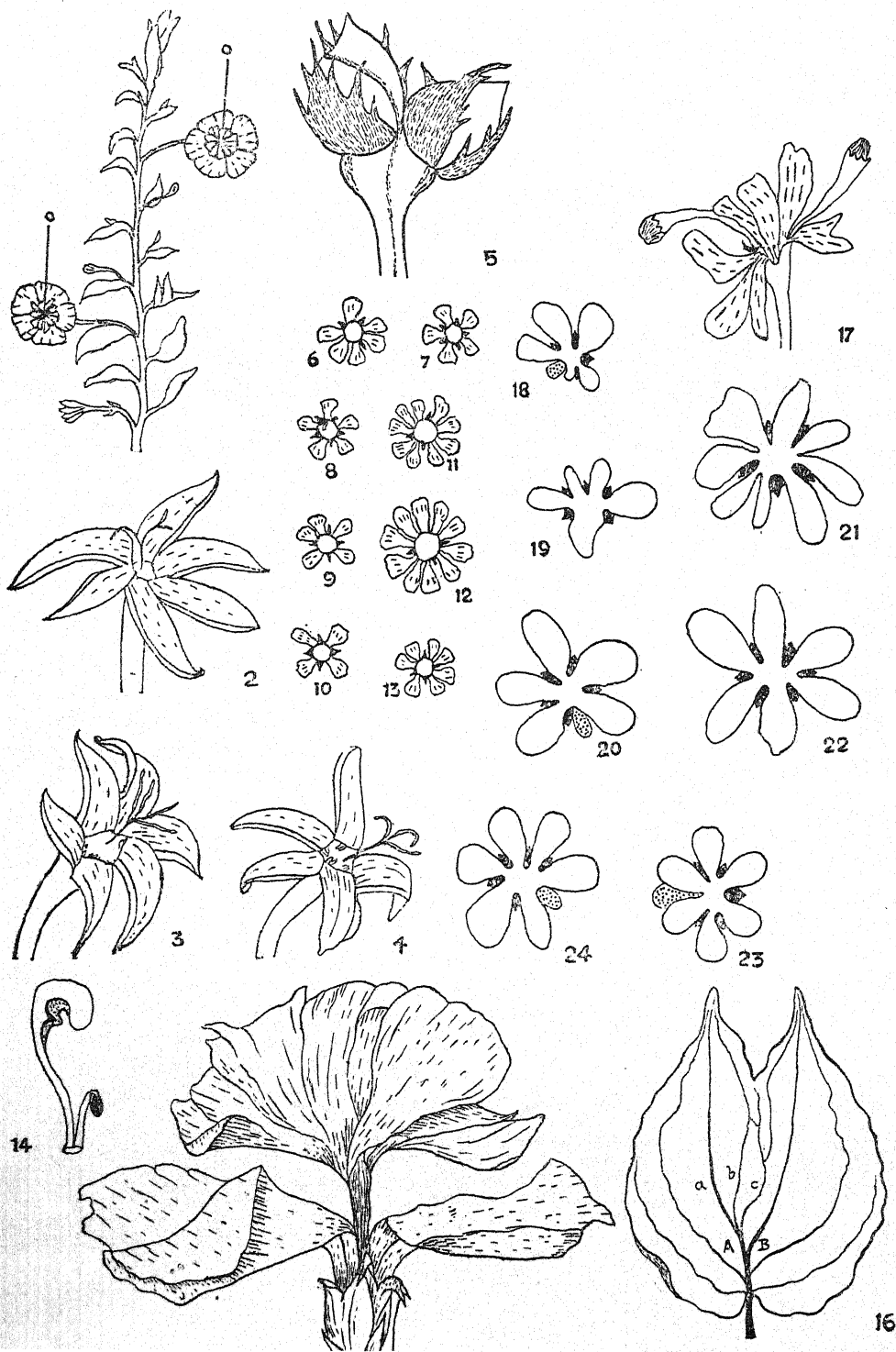
***Vitis vinifera* L.**

Seringe (6) long ago described in this species anomalies of the inflorescence which involved the fusion of two racemes ending in a tendril and Jaeger (2) mentioned peculiar raceme formation.

The specimens collected by me, however, consist in the flattening of the peduncle with ridges and furrows. It is interesting to note that in one case the flattened peduncle had curved round in the form of a loop, like that seen in *Crotalaria juncea* Linn. (8).

***Impatiens Balsamina* L.**

In this species several plants were met with in which the vegetative axes (partially or wholly) had become very much flattened (Fig. 43) recalling that of *Ranunculus acer* (14). The leaves, lateral branches and flowers, however, were borne in a normal manner.



A cross section through the flattened axis looks narrowly elliptical (Figs. 30-31) comparable to that of *Campanula media* (14). The cortex is very narrow and there is abundant secondary growth in the vascular cylinder. The pith on either side is hollow due to rupture of the tissue in sectioning. It is interesting to note that the trachae are of the liane-type.

Elaeocarpus ganitrus Roxb.

Double seeds of this species are rare and are at the same time very sacred with the Hindus. Some time ago, one hundred and eight such seeds were found in possession of a tobacco-merchant of Barh (Bihar) who had bought them at a rather high price from certain nomad hill *sadhus*.

The double seed is provided with two hilum-scars. The seeds are fused to each other laterally like *Areca catechu* L. (9), else they were found to be quite normal.

Gossypium

Variability in leaf is a common feature of the genus *Gossypium*. Tropea (4) has fully described this in the case of *Gossypium peruvianum* Cav. Certain other abnormalities hitherto unrecorded, are described below.

Gossypium neglectum Todaro: One of the commonest anomaly in this species is a peculiar humping of the boll on a side (Fig. 34). Then there is a less common case in which the boll together with its calyx-cup and epicalyx, is normally situated but the abnormality consists in the presence of a fourth epicalyx confluent with the calyx-cup by one of its margins (Fig. 35) only.

Of rather rare occurrence is the increase of floral parts in which a flower is found to consist of four very big well developed

Fig. 1: *Evolvulus alsinoides* Wall., a branch with two flowers showing petaloid out growths (o) in the corolla-tube. ($\times 1.4$); Figs. 2-4: *Morinda tinctoria* Roxb., abnormal flowers showing variation in the number of petals and stigmas. ($\times 1$); Fig. 5: *Rosa damascena* Mill., a triplet flower. ($\times 1$); Figs. 6-13: *Mangifera indica* L., show the variation of sepals (heavy black) and petals (lightly hatched) both in number and arrangement of parts. ($\times 1$); Fig. 14: *Dalbergia Sissoo* Roxb., an abnormal peculiarly hooded tenth stamen. ($\times 1.4$); Fig. 15: *Hibiscus Rosa-sinensis* L., a complete petaloidy of the staminal column. ($\times 1$); Fig. 16: *Piper Betle* L., a double leaf with two tips. ($\times 2/3$); Fig. 17: *Quisqualis indica* L., represents an extreme case of a fasciated flower with two normal flower-buds borne on the rim of the calyx-tube. ($\times 2/3$); Figs. 18-24; *ibid*, show variation in the number of sepals and petals; true sepals are represented black, the petaloid ones dotted and true petals blank. ($\times 2/3$).

leafy epicalyx (Fig. 33) and nine petals (Fig. 32); the staminal column and ovary are also much bigger than those of normal flowers. A section through the ovary shows double the number of loculi as compared to a normal ovary. The number of floral parts suggests a homologue of two normal flowers comparable to the condition seen in *Quisqualis indica* L. (7).

Gossypium indicum var. *malvensis*: By far the greatest variety of abnormalities is found in this species extensively cultivated in Central India.

In an abnormal flower, the epicalyx and other floral parts were found to be quite normal but due to phyllody, the sepals had grown out into several leafy expansions (Figs. 36-37) suggesting the presence of a second whorl of epicalyx.

Cases of adnation of the flower pedicel or the petiole with the main axis (Fig. 38) to a considerable distance are rather of common occurrence. A section through the fused region shows two separate steles completely surrounded on all sides by a common epidermis and cortex (Fig. 38a).

Flowers otherwise quite normal, often have two of their epicalyces represented by big leafy structures situated opposite each other, while the third, much smaller, is fused with the calyx-cup (Fig. 41).

Figure 39 represents a case having an extra leafy sepal adnate to the calyx-cup, the other floral parts being normal.

In another abnormal case there was only one epicalyx, the other two having aborted. One of the teeth of the calyx-cup was elongate. An interesting feature was the occurrence of an extra sepal on the inner side of the calyx-cup with which it was adnate (Fig. 40).

Figure 42 shows a case where six petals were found instead of the normal five. These were much too small and strap shaped; else the other floral parts were quite normal.

***Hibiscus Rosa-sinensis* L.**

In the case observed, the whole of the staminal column had become petaloid such that there was absolutely no vestige of any stamen (Fig. 15). Of greater interest, however, was the plant, which during the two years that the observations were continued, was never found to bear normal flowers. In this regard the present observation differs from all the previous observations (4).

***Quisqualis indica* L.**

The only recorded abnormalities in this species are those of a forked-lamina (4) and fasciation of flowers (7). Some time ago a number of abnormal flowers collected from Jehanabad railway station (E. I. Ry.) were examined. Most of them displayed considerable variation and petaloidy of sepals and stamens.

Figures 18-20 represent specimens with normal number of floral-parts; but in Fig. 20 only half of one of the sepals had become petaloid; in Fig. 18 one of the sepals had become wholly petaloid in addition to the marked size variation of the petals; in Fig. 19 the petals were variable in shape and size and their arrangement was rather peculiar.

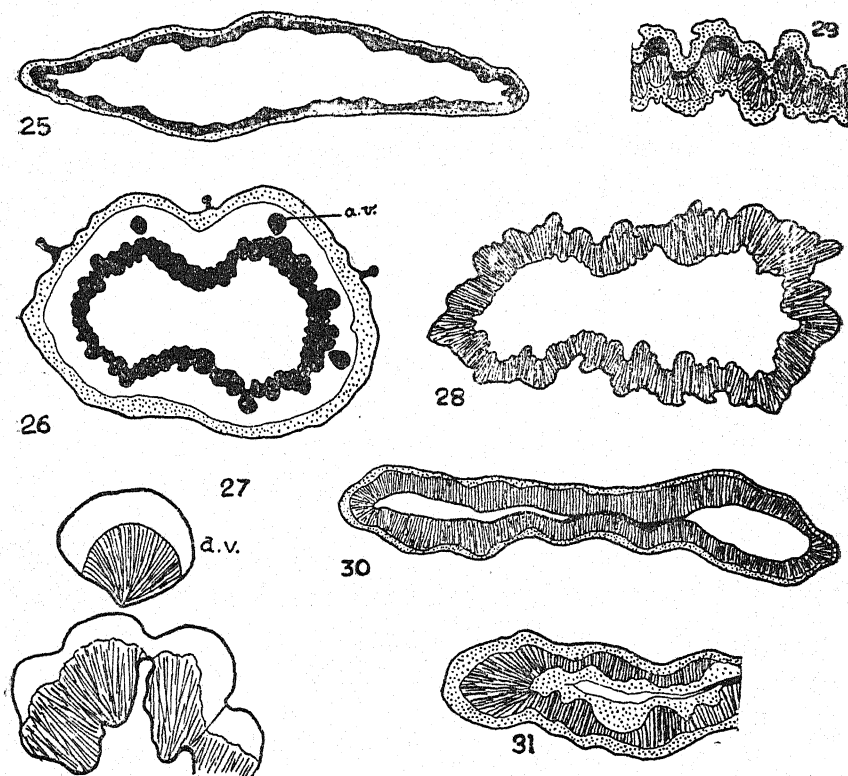


Fig. 25: *Lycopersicum esculentum* Mill.; cross section through a flattened stem showing the narrow cortex (dotted), stele (black) and pith (blank). ($\times 5$); Fig. 26: *Rosa damascena* Mill.: cross section through the composite peduncle showing the outer ring of accessory vascular bundles (a. v.) and the peripheral belt of streome (dotted). ($\times 10$); Fig. 27: *ibid*, a portion of the stele together with an accessory strand highly magnified. ($\times 80$); Fig. 28: *Tagetes erecta* L., transverse section of the flattened stem showing ridges and grooves with a large pith (blank). ($\times 5$); Fig. 29: *ibid*, a portion of the stele magnified showing the fibro-vascular bundles (hard bast represented heavy black) embedded in a thick-walled tissue (dotted). ($\times 10$); Fig. 30: *Impatiens Balsamina* L., transverse section of the flattened stem showing the narrow cortex (dotted) and hollow pith on the sides ($\times 5$); Fig. 31: *ibid*, a portion of the latter magnified showing the stele (hatched), ground tissue (dotted), pith hollow. ($\times 10$).

Then we come across flowers which are actually hexamérous (Figs. 22-24): **22** represents a typical hexamérous flower; **24** is like **22** but one of the sepals has become wholly petaloid; **23** is like **24** except that the petaloid sepal has attained a size equalling a normal petal.

The specimen represented by Figure 21 is actually pentamérous but has an increased number (eight) of petals. This is due to complete chorisis of three petals of the whorl.

Very peculiar indeed is an abnormal fasciated flower (Fig. 17) in which no less than 4 flowers are involved in the process. The lower calyx-tube is formed by the fasciation of two flowers. This is substantiated by the increased number of floral parts and the presence of two gynaeceia. But the most interesting feature is the occurrence of two flower-buds, one on either side on the rim of the calyx-tube exactly in a position where one would ordinarily expect petals. These two flowers appear to be normal in every way.

Psidium Guyava L.

The only abnormality recorded in this genus is apparently that by Braun (1) who observed gnarl formation on the point of a strong branch in *Psidium pomifera*.

In *Psidium Guyava* ascidial leaves — hitherto un-recorded — of various shapes have been met with. The water holding capacity of each on examination has been found to vary considerably — depending on the size and depth — from 5 cc. to 20 cc.

Evolvulus alsinoides Wall.

Abnormal flowers were collected from plants growing wild in nature. The anomaly consists in the presence of a number of petaloid outgrowths in the wider portion of the throat of the corolla-tube without in the least disturbing the juxtaposition of the epipetalous stamens. The outgrowths are about a dozen in number and are strap-shaped provided with a vein. The whole thing offers an impression of a double flower (Fig. 1).

Lycopersicum esculentum Mill.

Several abnormal plants were observed, in which all the vegetative as well as reproductive axes had become very much flattened (Fig. 44) looking like the fasciated stem of *Ranunculus acer* (14). A cross section through the flattened axis is more or less elliptical with attenuated ends (Fig. 25). The pith is very broad while on the other hand the cortex is narrow. The xylem though meagrely developed, is composed of trachae of the lianetype (cf. *Rosa damascena*).

***Ercanthemum atropurpureum* Hort.**

The abnormal leaves observed in this species are forked, and like *Lonicera perclymenum* (14) display all stages in the forking of the mid-rib and the lamina.

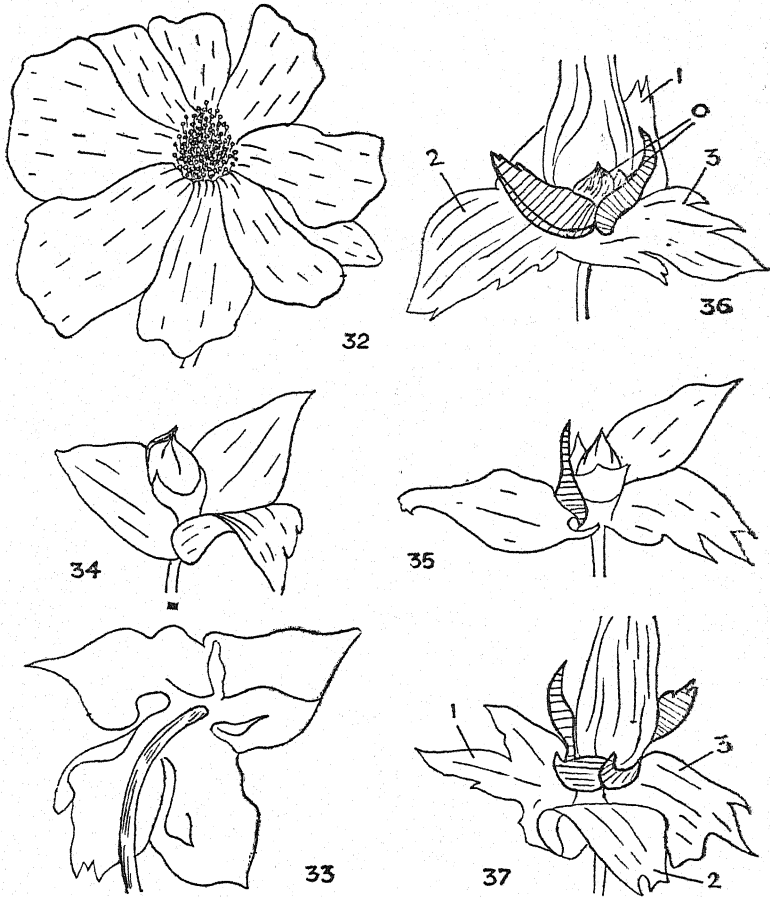


Fig. 32: *Gossypium neglectum* Todaro, an abnormal flower showing nine petals and a bigger staminal column. ($\times 2/3$); Fig. 33: *ibid.*, the same abnormal flower as seen from the bottom showing four epicalyx. ($\times 2/3$); Fig. 34: *ibid.*, a humped boll ($\times 2/3$); Fig. 35: *ibid.*, a case showing a fourth epicalyx (hatched) adnate to the calyx-cup by one of its margins ($\times 1/4$); Figs. 36-37: *G. indicum* var. *malvensis*; two views of the same specimen showing the development of a number of leafy sepals (hatched), 1, 2, 3, represent the three normal epicalyx, 0 represents two phyllodous sepals which are on the other side and hence not seen in Fig. 37. ($\times 2/3$).

***Morinda tinctoria* Roxb.**

While engaged on a study of the pollination of this interesting Rubiaceae tree (12, 13), certain abnormal flowers were observed. The commonest anomaly was that of the presence of tri-fid stigma in various degrees of furcation (Figs. 2, 4) instead of the normal two. These flowers were otherwise quite normal except in one case which had six petals (Fig. 2). Rarely the stigma may be even tetra-fid (Fig. 3); in such cases, however, flowers invariably always possessed an increased number of petals and stamens.

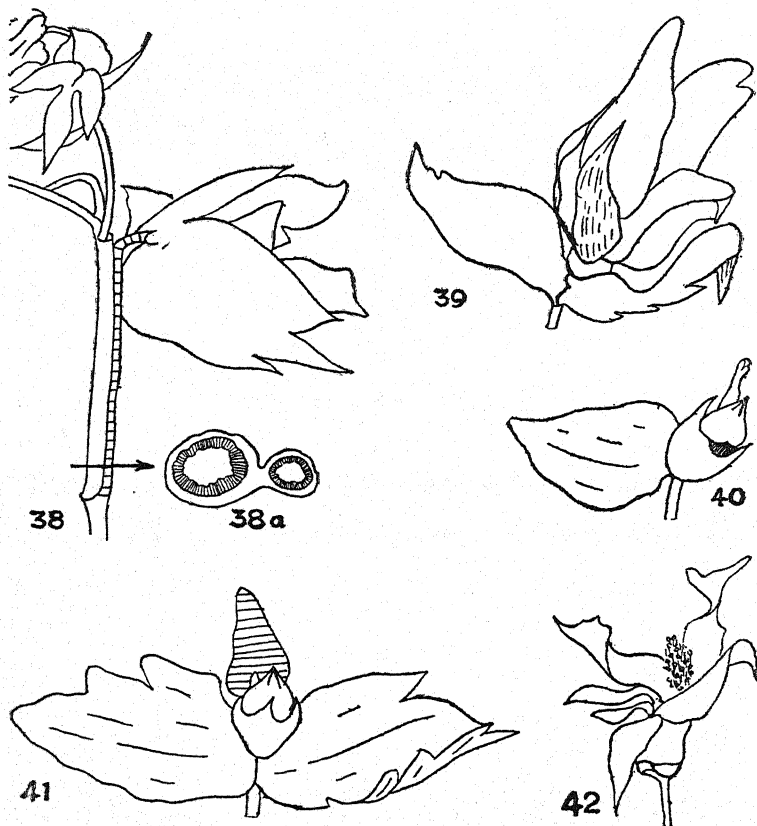


Fig. 38: *Gossypium indicum* var. *malvensis*, adnation of the flower pedicel (hatched) to the stem. ($\times 3/4$); Fig. 38 a: *ibid*, a cross section through the fused portion representing the stem on the left and the pedicel on the right connected to each other by a "neck" of tissue. ($\times 6$). Fig. 39: *ibid*, an abnormal flower with an extra leafy sepal (hatched) adnate to the calyx-cup ($\times 3/4$); Fig. 40: *ibid*, a case with only one epicalyx an elongate calyx-teeth and an extra sepal (hatched) on the inner side of the calyx-cup. ($\times 3/4$); Fig. 41: *ibid*, shows two big nearly opposite epicalyx and the third (hatched) adnate with the calyx-cup. ($\times 3/4$); Fig. 42: *ibid*, a specimen with six petals of varying size. ($\times 3/4$).

Tagetes erecta L.

In this species like *Lycopersicum esculentum* (p. 320), the vegetative as well as reproductive axes of an entire plant, have undergone a complete flattening and the surface is provided with ridges and grooves (Fig. 45). A cross section (cf. *Campanula media* (14)) through such an axis is broadly elliptical with corrugations (Fig. 28). The pith is large and the cortex comparatively narrow. The stele is embedded in a belt of tissue constituted of thick-walled cells (Fig. 29). Here also the trachae are of the liane-type. The capitula borne on such plants are all normal.

Summary

This paper comprises a study of abnormalities in seventeen species belonging to fifteen representative families of angiospermous plants:

- I. The leaf:
 - (a) *Piper Betle* L.: double leaves with two tips.
 - (b) *Psidium Guyava* L.: ascidial leaves.
 - (c) *Eranthemum atropurpureum* Hort.: double leaves with 2 tips.
- II. The stem:
 - (a) *Gossypium neglectum*: adnation of the petiole or the flower pedicel to the stem.
 - (b) Flattening of the stem in *Impatiens Balsamina* L. *Lycopersicum esculentum* Mill. and *Tagetes erecta* L.
- III. The flower:
 - (a) *Rosa damascena* Mill.: a triplet.
 - (b) *Dalbergia Sissoo* Roxb.: transformation of the tenth stamen into a peculiar hooded structure.
 - (c) *Magnifera indica* L.: variation in number and alternation of the calyx and corolla.
 - (d) *Vitis vinifera* L.: flattening of the peduncle.
 - (e) *Hibiscus Rosa-sinensis* L.: a complete transformation of the staminal column into petals.
 - (f) *Gossypium neglectum* Todaro: a flower showing double the number of floral parts and occurrence of extra sepals, petals and epicalyx.
 - (g) *G. indicum* var. 'malvensis': leafy sepals; two big epicalyx opposite each other and the third much smaller adnate with the calyx-cup; an extra leafy sepal adnate to the calyx-cup; presence of only one epicalyx in a flower with an extra sepal; presence of an extra petal.

- (h) *Quisqualis indica* L.: a very peculiar fasciation of four flowers into one; various stages in the petalody of sepals; chorisis of petals.
- (i) *Evolvulus alsinoides* Wall.: presence of a number of petaloid out-growths in the wider portion of the throat of the corolla-tube.

IV. The fruit:

- (a) *Gossypium neglectum* Todaro: humped bolls.

V. The seed.

- (a) *Elaeocarpus ganitrus* Roxb.: double seeds.

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Explanation of Plate

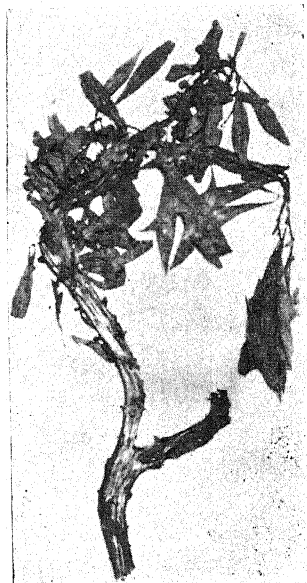
- Fig. 43. *Impatiens Balsamina* L.
- Fig. 44. *Lycopersicum esculentum* L.
- Fig. 45. *Tagetes erecta* L. shows flattening of the vegetative axis. The leaves and flowers borne are normal. (× 5).



43



44



45



ON THE PEG OF THE SEEDLINGS OF *CUCURBITA MAXIMA* DUCHESNE.

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Received for publication on 25th March, 1935.

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Introduction

F. Darwin and E. H. Acton (6) say "When a seed of *Cucurbita ovifera* is allowed to germinate in normal conditions the well-known peg or heel is developed on the physically lower side of the radicle. But if the seeds are kept in slow rotation on the klinostat until they germinate, the peg is not developed laterally, but like a frill all round." They deduced from the formation of a frill, that "even in a slowly moving klinostat the stimulus is perceived but is equally distributed."

In several books (1, 8, 9), the diageotropic peg has been described and sketched, but nowhere any mention has been made as to what happens when the seed is germinated with its

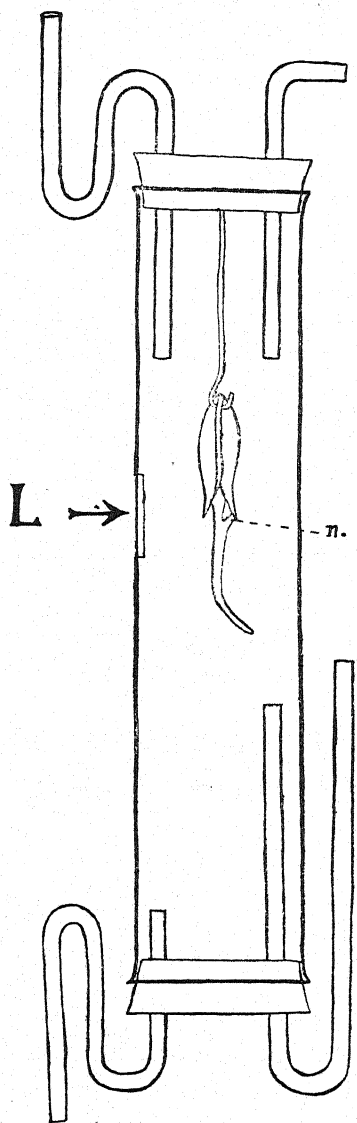
micropyle downwards and also whether light has any action over peg formation. With a view to finding out the effect of the position of the seed on the peg formation, a number of seeds were germinated in various positions on a cork slab covered with blotting paper and floated on water. In seeds placed horizontally, flat or edgewise, and seeds placed vertically with their micropyle upwards, the radicle bent down and peg formed on the concave side. In seeds placed vertically with their micropyle downwards, the radicle grew straight down and pegs developed always nearest the slab. It was assumed that, when no particular side of the radicle was influenced by gravity but one side was darker (the side nearest the slab in this case), a negatively heliotropic peg develops on the less lighted side. To test this assumption the following experiments with seeds of *C. maxima* Duchesne, were performed.

Experiments

Gravity—In a pot containing sand (i) some seeds were sown broadside flat (axis horizontal), (ii) some seeds edgewise (axis horizontal), (iii) some with micropyle upwards (axis vertical) and (iv) some with micropyle downwards (axis vertical). The seedlings, when 94 hours old, were photographed [Plate I, Figs. (i) —(iv)]. In (i), (ii) and (iii) it was found that the radicle had bent and grown downwards, a peg had formed on the physically lower side (concave side) at the base of the hypocotyl and by the straightening of the hypocotyl loop, the cotyledons had come up, the testa having been pegged down in the soil. In (iv) a *frill* was found instead of a peg. The development of a peg on the physically lower side in (i), (ii) and (iii) is due to the influence of gravity and it is positively diageotropic. *A frill is formed instead of a peg when the radicle grows in the position of geotropic equilibrium.*

A number of seeds were suspended with their micropyle downwards in a moist glass chamber. As in Plate I, Fig. (iv), *frills* were formed in all of them.

Light—Two glass tubes with corks, having inlets and outlets for both air and moisture on both the sides, were taken. Two seeds were taken, each of which was hung from a cork with its long axis vertical and micropyle downwards inside a moist tube. Both the tubes were darkened by covering with black paper, but in one of them light was allowed through a long narrow chink opposite the seed near its micropyle (Text-fig. 1). After three days it was found that in the seedling germinated within the fully covered tube, a *frill* had developed (Plate II, Fig. 1), whereas in the seedling getting light on one side, only a *peg* had developed on the dark side (Plate II, Fig. 2). Hence it may be concluded that *the peg is also negatively heliotropic.*



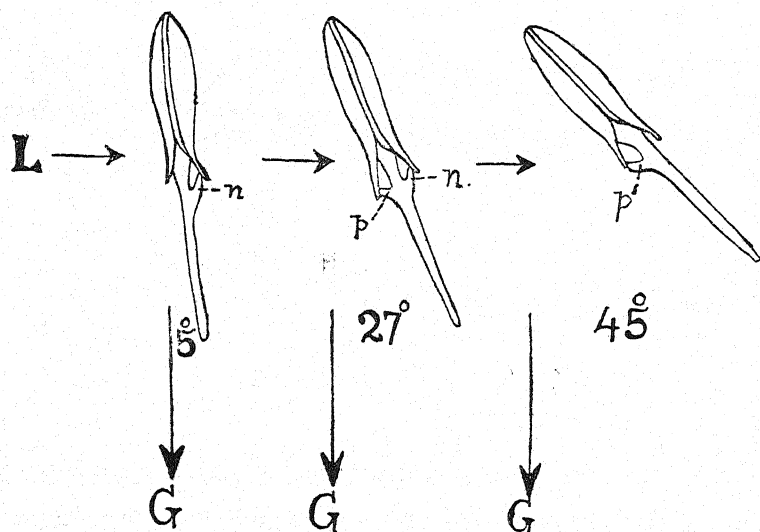
TEXT-FIG. 1.

The whole tube was covered with black papers excepting the small slit indicated by the arrow.

n = negatively heliotropic peg.

Light and Gravity—A number of seeds were taken, each of which was attached by a wire at its round end to the cork of a tube. The long axis of the seed inside the tube was kept parallel to its sides. All the tubes were darkened by covering with black paper having a narrow chink on one side only opposite the seeds, as in Text-fig. 1. The tubes were placed inclined at various angles to the vertical and consequently the long axes of the seeds whose micropyles were downwards, were inclined to the vertical. The chinks were on the lower side of the tubes. So the lower sides of the seedlings were influenced by both light and gravity. Only daylight was utilised for the purpose. In the seedling the long axis of which was slightly inclined (about 5°) the intensity of gravity was slight and that of light great. Hence in this seedling the *peg* was found on the *dark side* (the *upper side*). In the control experiment where the axis of the seedling was so inclined but equally illuminated on all sides, a *peg* had developed on the *lower side*. As the angle between the axis and the vertical increases, the intensity of gravity also increases and that of light decreases. So in the seedling, the axis of which was inclined at an angle of about 25° , where both light and gravity influenced nearly equally, two *pegs* developed, one positively diageotropic peg on the physically lower side due to the influence of gravity and another negatively heliotropic peg on the dark side (also the upper side).

due to that of light (Plate II, Fig. 4). In the control experiment where the axis of the seedling was so inclined but equally illuminated on all sides, only one positively diageotropic *peg* developed on the physically lower side (Plate II, Fig. 5). In the seedling the axis of which was inclined at an angle of about 45° , where the influence of gravity was greater than that of light, only one positively diageotropic *peg* developed on the physically lower side. (Text-fig. 2).



TEXT-FIG. 2.

Direction of light indicated by transverse arrows = L.

Direction of gravity indicated by vertical arrows = G.

n = negatively heliotropic peg.

p = positively diageotropic peg.

There appears to be a mathematical relationship between the influences of light and gravity on the formation of peg. When gravity and light act in lines perpendicular to each other, the intensity of gravity stimulus varies as $\sin \theta$ and that of light as $\cos \theta$, θ being the angle between the axis of the seedling and the vertical. The intensity also varies as the presentation time. The perception regarding peg formation has been found to last for a long time, for about 48 hours. As only daylight was utilised, it may be presumed that gravity has acted for time "t"

and light for " $\frac{1}{2}t$ ". Accordingly the following conclusion may be arrived at:—

—	Gravity.	Light.	Ratio (G/L)	Result.
$\theta=5^\circ$	Sin 5° . t	Cos 5° , $\frac{1}{2}t$	0.087/0.498	(i) no positively dia-geotropic peg, but (ii) a negatively heliotropic peg only.
$\theta=27^\circ$	Sin 27° . t	Cos 27° , $\frac{1}{2}t$	0.453/0.446 nearly equal	(i) a positively dia-geotropic peg and (ii) a negatively heliotropic peg.
$\theta=45^\circ$	Sin 45° . t	Cos 45° , $\frac{1}{2}t$	0.707/0.353	(i) a dia-geotropic peg only, (ii) no negatively heliotropic peg.

(cf. Text-fig. 2.)

According to Jost (7) "The chief difficulty in all such experiments lies in obtaining equally great excitations by different stimuli. The goal aimed at is perhaps quite unrealisable, if *different and non-comparable excitations* correspond to *different stimuli*."

But in this experiment it is quite clear that "every effective reaction inhibits every other reaction" (10) and also that when both the forces are equal two responses are made.

Centrifugal Force—(i) Some seeds were attached radially to a vertical corkplate with their micropyle outwards and (ii) some seeds tangentially at its side. The plate was constantly kept moist and rotated by an electric motor about 11 times per second. Thus the axes of the germinating seedlings were subjected to a centrifugal force of a considerable magnitude. In the first case the radicle elongated outwards in the direction of the force and a frill, though small, was found to have developed all round. In the second case the radicle bent and elongated outwards at right angle to the axis of the seed and a peg was found on the concave side. The frill in the first case may be compared with the frill in the seedling in which the radicle was allowed to grow in a position of geotropic equilibrium and the peg in the second case may be compared with that which was formed in the seedling germinated broadside flat. The only difference is that in this experiment gravity was substituted by centrifugal force.

Clinostat—The experiment with the clinostat as mentioned in the "Practical Physiology of Plants" by F. Darwin and

E. H. Acton, was repeated by the author by germinating seeds with their axes horizontal on the vertical plate of the instrument which was completing a revolution every fifteen minutes. As was found by Darwin and Acton, a frill developed all round and this was due to summation of gravity stimuli all round.

Decapitated Radicle—In one seed, pinned with its micropyle downwards, to the side of the cork-slab floated on water, the radicle did not grow. But after some days the hypocotyl elongated and a frill was found, whereas in other normal seedlings so placed, a peg developed on the darker side. This was suggestive of the peg being influenced through the radicle.

So a number of seeds were germinated in sand in a position of geotropic equilibrium and after about 40 hours when the radicle had grown about 5 mm. in length, they were taken.

Two such seedlings were taken. In one of them the tip was removed to about 3 mm. Both of them were placed with radicles vertically downwards inside dark tubes allowing light through one side only (cf. Text-fig. 1). In the entire seedling a negatively heliotropic peg developed on the dark side (Plate II, Fig. 2) whereas in the seedling the root-tip of which had been decapitated, a frill developed all round (Plate II, Fig. 3). Therefore it may be concluded that *the light stimulus leading to peg formation is perceived through the root-tip*.

Several other seedlings were taken and their root-tips were decapitated to various lengths. Some were placed horizontal and some with micropyle upwards. The radicle did not grow nor bent, but the hypocotyl curved down in both the cases and pegs formed on the lower side (Plate II, Figs. 6 and 7). Before decapitation of the tips the seedlings were growing in a neutral position and, had the previous influences, if any, persisted, frills would have formed. Therefore it may be concluded that *the root-tip is not the only perceptive region for gravity stimulus leading to peg formation*.

Structure of the Peg

In the hypocotyl, all the cells at its base are meristematic at the time when the peg makes its appearance. The peg or frill as observed externally, develops upwards making a pocket with the axis and when fully formed opens outwards to make a ridge. A few cells of the dermatogen and of the periblem are concerned in the formation of the peg which bulge upwards and increase in number by mitosis. On the opposite side of the axis corresponding to the region of the peg, there is no bulging and no cell division. Vascular elements have never been found in a peg.

Biological Significance

When a seed of *C. maxima* is germinated with micropyle vertically downwards, the radicle grows down straight in a position of geotropic equilibrium, a frill is formed at the base of

the hypocotyl, the hypocotyl tends to form a loop and attempts to bring the cotyledons out of the testa, but in the meantime the frill loses its hold on the testa and comes out of it and then by further growth the hypocotyl straightens and the testa is borne above the soil with the cotyledons inside it. It takes about a week for the cotyledons to throw out the testa by variously twisting themselves. If the seed is germinated in any other position, the radicle grows and bends downwards, a peg is formed on the physically lower side and catches hold of the nearest lobe of the testa, the hypocotyl then forms a loop presenting the concave side to the peg and by its straightening the cotyledons come out of the testa which remains pegged in its previous position. The cotyledons on coming out of the testa turn green and begin to prepare food [cf. Figs. (i a) and (iv a) in Plate I]. By the formation of a positively diageotropic peg the cotyledons move in the opposite direction towards the surface of the soil and get a chance to reach light. And by the formation of a negatively heliotropic peg the cotyledons move towards light.

Discussion

The growth of the peg or of the frill at the base of the hypocotyl is controlled by certain factors. The cells in that region all round or on one side are stimulated to divide and grow for the purpose. They are not directly stimulated by light or gravity but through the radicle or hypocotyl. It is highly probable that due to light or gravity some hormones (2), growth substances (3) or growth regulators, G. R. (4), are produced at the tip cells, which when conducted reach the hypocotyl and inhibit or induce growth there.

According to the author, at the root-tip one G. R., positive in case of gravity and negative in case of light, is produced in the stimulated half and that the same substance acts antagonistically on the other side. Here the two halves of the responsive area may be compared with two antagonistic muscles and for co-ordination each responds antagonistically to the other as a result of the same stimulus (5). Or it may be conceived that a cell or an organ possesses two opposite transverse poles (7) and that any action on a pole induces an opposite reaction of the same intensity in the other pole.

When the axis of the *C. maxima* seedlings is in a position of equilibrium or rest, the outgrowth takes place all round at the base of the hypocotyl.

When the axis is in any other position a + G. R. is produced in the lower half of the tip which induces growth on the lower side of the hypocotyl and inhibits it on the other side.

When the axis is vertical and one side is lighted, in the exposed half of the seed, a - G. R. is produced which

inhibits growth on that side but induces it on the opposite side.

When a side of the axis is stimulated both by gravity and light, both + and - G. R.'s are produced on the exposed side but their strength varies according to the intensity of the forces and the stronger one is more effective. When they balance each other, two pegs are formed.

Summary and Conclusion

1. A *peg* or a *frill* grows at the base of the hypocotyl in the seedlings of *Cucurbita maxima* Duchesne, during the early period of their germination. A *frill* grows when the axis of the seedling is in a position of equilibrium regarding both gravity and light.

2. When a particular side of the axis is influenced by gravity only, by being the lower side, then the outgrowth is induced on that side only but inhibited on the other side, resulting in a positively diageotropic *peg*.

3. When a particular side of a vertical radicle is influenced by light, the outgrowth is induced on the opposite side only but inhibited on the illuminated side resulting in a negatively heliotropic *peg*.

4. When gravity and light act in lines perpendicular to each other, on a particular side of the radicle which is inclined at a certain angle with respect to the vertical, two opposing influences come into play but the one which is greater in intensity evokes the outgrowth. The intensity of the gravity stimulus varies as $\sin\theta$ and that of light as $\cos\theta$, θ being the angle between the inclined axis and the vertical. The intensity of a force is also proportionate to the time during which it acts and every effective reaction inhibits every other reaction. When both the forces are equal, two *pegs* grow on opposite sides.

5. When the radicle is slowly rotated horizontally on a clinostat, due to summation of gravity stimuli, a *frill* is formed all round.

6. When the radicle grows straight with the axis, due to centrifugal force, a *frill* is formed.

7. When the radicle bends at right angles, due to centrifugal force, a *peg* is formed on the concave side.

8. When the radicle grows vertically downwards with the tip removed and light is applied from one side, no negatively heliotropic *peg* but a *frill* is formed. So the tip of the radicle is the perceptive region for the light stimulus regarding *peg* formation.

9. When the radicle grows horizontal with its tip removed a positively diageotropic *peg* is formed. The decapitated root does not bend but the hypocotyl curves down and the peg forms on the concave side. So probably the root tip is not the only perceptive region for the gravity stimulus regarding peg formation.

The author expresses his indebtedness to Prof. P. Parija, M.A. (Cantab), for his useful suggestions and generous encouragement, and to Mr. P. C. Mallik, M. Sc., for preparing certain photographs herein.

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Explanation of Plate I

Fig. (i) *Cucurbita maxima* Duchesne: seed germinated broadside flat with its axis horizontal.
p = positively diageotropic peg. $\times 3/4$.

Fig. (ia) *Idem*: 6 days old. $\times 3/4$.

Fig. (ii) *Idem*: germinated edgewise with its axis horizontal. p = positively diageotropic peg. $\times 3/4$.

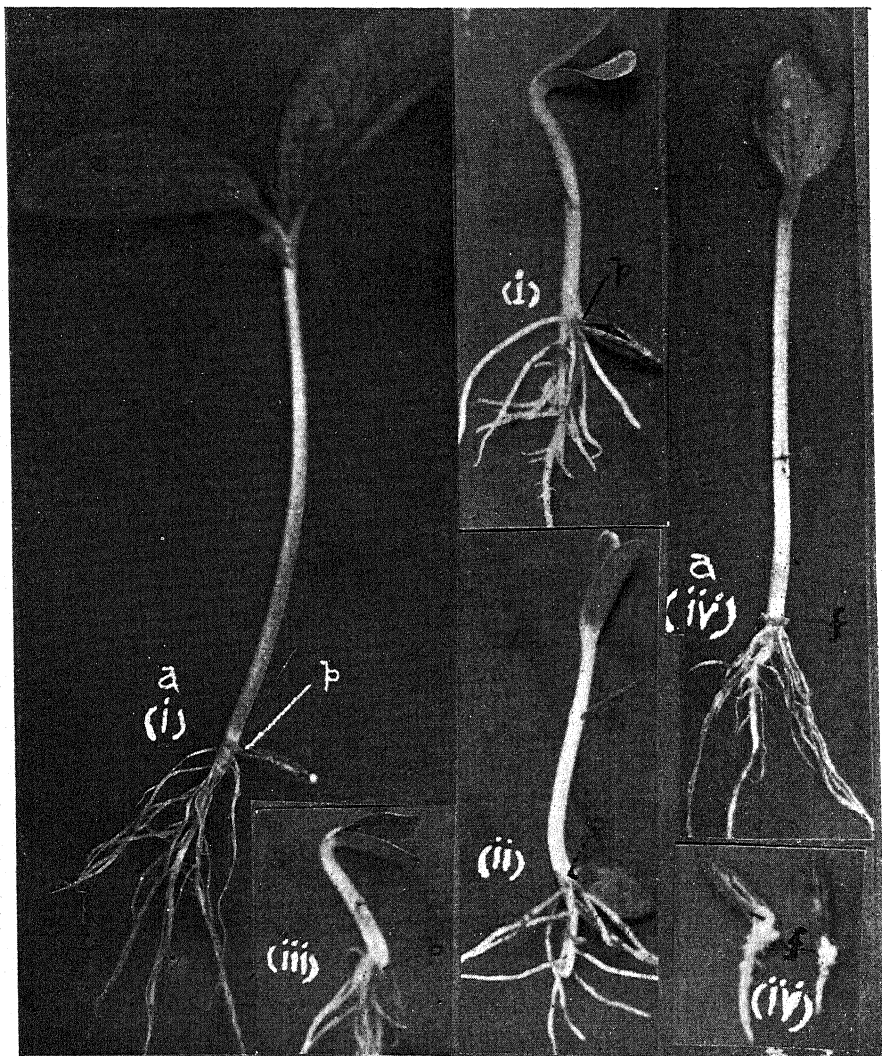
Fig. (iii) *Idem*: germinated vertical with micropyle upwards. p = positively diageotropic peg. $\times 3/4$.

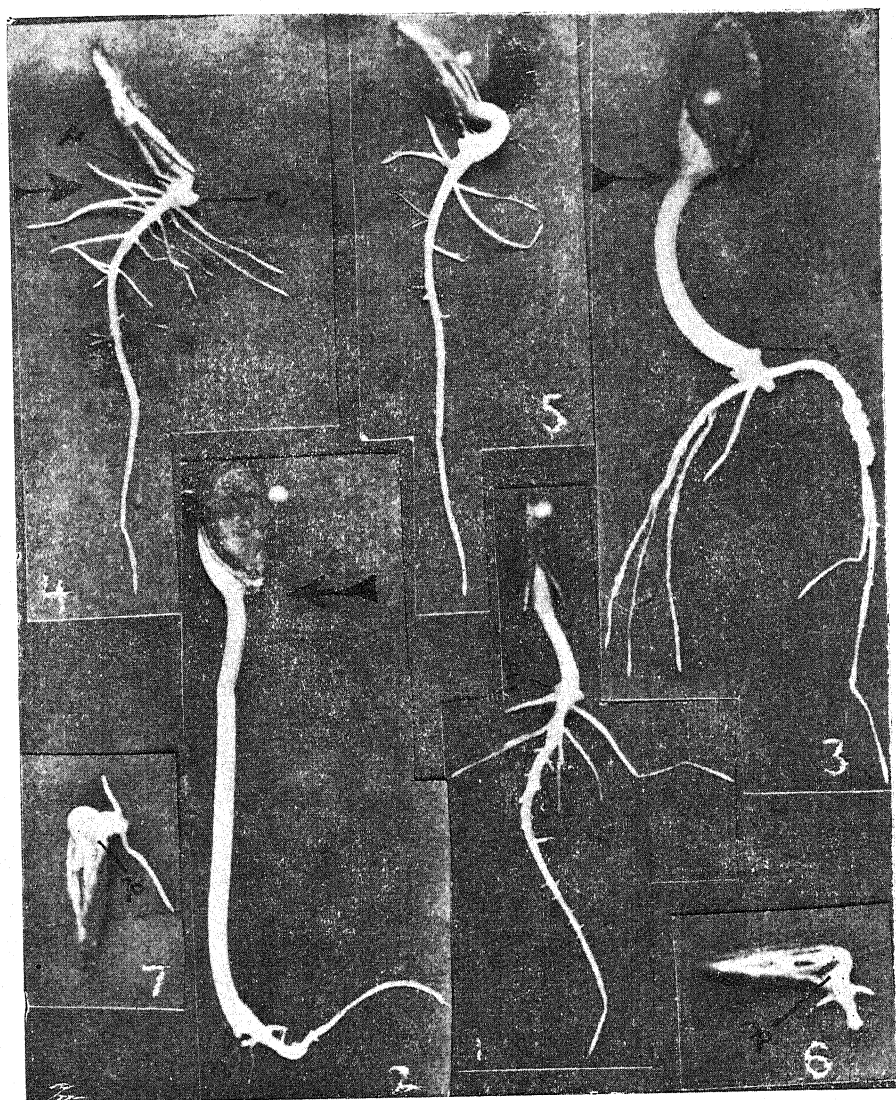
Fig. (iv) *Idem*: germinated vertical with micropyle downwards. f = frill. $\times 3/4$.

Fig. (iva) *Idem*: 6 days old. $\times 3/4$.

Explanation of Plate II

- Fig. 1. *Cucurbita maxima*, Duchesne: seedling grown vertical with micropyle downwards in a dark-chamber. f = frill. $\times 1$.
- Fig. 2. *Idem*: grown vertical with micropyle downwards in a dark chamber and lighted from a side. n = negatively heliotropic peg. Direction of light indicated by an arrow. $\times 1$.
- Fig. 3. *Idem*: with root-tip decapitated. f = frill. $\times 1$.
- Fig. 4. *Idem*: grown with its micropyle downwards and its axis at an angle of about 25° with respect to the vertical in a dark chamber lighted during the day time from the lower side. p = positively diageotropic peg; n = negatively heliotropic peg. Direction of light indicated by an arrow. $\times 1$.
- Fig. 5. *Idem*: in light. p = positively diageotropic peg. $\times 1$.
- Fig. 6. *Idem*: grown broadside flat and horizontal with the root-tip decapitated. p = positively diageotropic peg. $\times 1$.
- Fig. 7. *Idem*: grown vertical with micropyle upwards and the root-tip decapitated. p = positively diageotropic peg. $\times 1$.





NOTE ON THE MOVEMENTS OF *BASELLA CORDIFOLIA* LAMK.

BY

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Communicated by T. C. N. Singh, Sabour.

Received for publication on 8th March, 1935.

Basella cordifolia Lamk., and some of the allied species generally grown in vegetable gardens, are, strictly speaking, not true climbers, but are of the nature of succulent stragglers and can easily be induced to twine up any suitably placed support.

In the monsoon of 1930 the writer observed a young plant of *Basella cordifolia* growing in his garden curving towards a support though the latter was about 3 feet away from it. This phenomenon seemed to suggest that the plant was capable of some peculiar perception of the support in its vicinity and of responding by certain growth-curvatures. The literature available to the writer failed to show record of any such phenomenon having been observed before and a few preliminary experiments were therefore undertaken in order to examine the phenomenon more closely.

In 1931, a row of seedlings was planted parallel to a wall about 3 feet away from it. When the plants were about a foot high, all of them were observed to curve and grow towards the wall, ultimately reaching it.

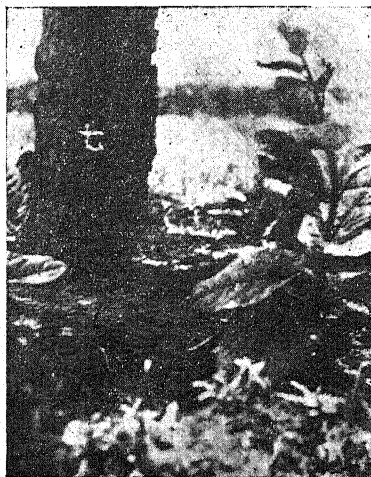
During the last season, a few more observations were made. Seedlings were planted in a row parallel to, and another row at right angles to, a wall facing south. Another row of seedlings was planted alongside another wall facing north. In all such cases the plants curved towards the wall. In the case of the plants near the south wall the plant nearest the wall was 3 and the farthest was 10' from the wall. At an early stage, the shoots were observed to curve towards the wall. The tendency became more pronounced as the plants grew older, till finally all the plants (5 in number) crept along growing directly towards the wall. At first, there were no other objects in the vicinity, but subsequently the bough of a

tree was placed slantingly against the wall, and shortly after, it was noticed that all the distantly situated plants changed their direction slightly and began to grow towards the branch.

This curious tendency was further put to test in a series of experiments in all of which the plants were situated in a circle round a central support, in one case a stout vertical wooden post (Fig. 1), in the second, a small tree (Fig. 2) and in the third, a brick gabion. In all cases, the plants were well removed from the central object, the distances being about 18 inches, $3\frac{1}{2}$ feet and 4 feet respectively. Briefly stated, in all these cases the plants behaved as they did in the earlier experiments. Almost all the plants gradually curved towards the central object, some more sharply than the others.



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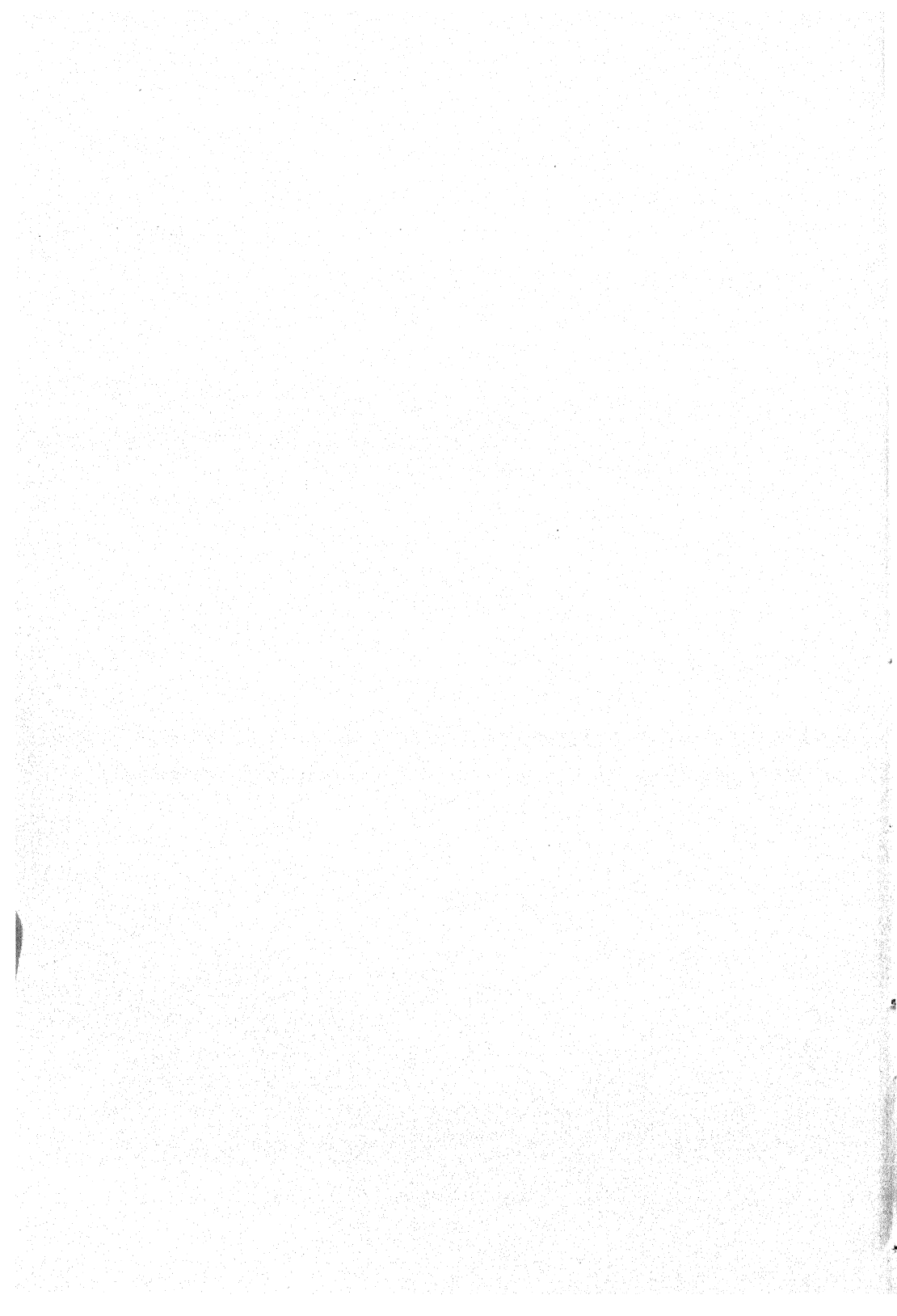
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A young plant of *Basella cordifolia* Lamk., growing towards an wooden post (w) in Text-figure 1; and another towards the trunk (t) of a small tree in Text-figure 2.

In fact, while the plants under normal and favourable conditions curved and grew in the manner described before, the behaviour of such of the others as had been adversely affected in respect of their normal curvatures through unfavourable environmental factors was particularly striking. For instance, in one or two cases, the young shoots were from the beginning blown away from the support by a continuous east-wind which prevented them from assuming the centripetal curvatures at an early stage like the other plants in the experiments. But convincingly enough, these plants, at a later stage, not only developed sufficient resistance to withstand the wind, but actually curved back again towards the support in spite of the wind.

A similar behaviour was also observed in another shoot which had been diverted from its original course by a current of flood water. When the water subsided, the growing shoot gradually curved again toward the central support from its new position.

Messrs. Rama Rao Panje, M. Sc., and B. C. Mitra, L. Ag., assistants in the Patna Experiment Station, rendered valuable assistance in carrying out the work.



RECENTLY INTRODUCED OR OTHERWISE IMPERFECTLY KNOWN PLANTS FROM THE UPPER GANGETIC PLAIN

BY

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Received for publication on 20th May 1935.

The area dealt with in the present communication includes the whole of the Upper Gangetic Plain extending from the Jumna south-eastwards along the Siwalik range of hills and the Sub-Himalayan tracts to the Gandak as well as Central India, Bundelkhand, the Malwa plateau and eastern Rajputana. It comprises, therefore, all the plains districts of the United Provinces of Agra and Oudh including those touching and extending to some extent into the foothills of the Himalaya but not the hill districts.

Such general works as the Flora of British India by Hooker and Indian Trees by Brandis deal with the vegetation of this area in general with the rest of the flora of India but it is dealt with in particular in Duthie's Flora of the Upper Gangetic Plain and of the adjacent Siwalik and Sub-Himalayan Tracts on which work our knowledge of the plants of this area is chiefly based. This work is constantly used by teachers and students of botany in our Indian universities while dealing with plants from the Upper Gangetic Plain both in the class-room and the field. It therefore appears desirable to place on record from time to time any notes or observations made in connection with it. It is hoped that these notes will be found useful in bringing the Flora up-to-date at the time of its completion and it is therefore with this object in view that the list is published. The list may, in a sense, be called a supplement to Duthie's Flora but it does not claim to be a complete one. For the benefit of those who may use this list I have added detailed descriptions of those plants which are not given in Hooker's Flora of British India and of which it may therefore be difficult or inconvenient to find descriptions.

A perusal of the pages that follow will show to what an amazing extent some plants indigenous to Tropical America, on finding a suitable habitat, are becoming established and naturalized in the region of the Upper Gangetic Plain,

To those who are interested in the progress of botany of our region I would like to make a few suggestions as to the manner in which they can assist. Most Floras are written from and based upon plant specimens which have been collected in the area dealt with, pressed and mounted and carefully preserved in herbaria for future reference. The addition of field notes relating to these plant specimens greatly increases their value, especially of such field notes like the habit and habitat of the plant, colour and texture of bark, colour of young foliage, flower and ripe fruit, etc., which cannot be seen in the herbarium specimens themselves. If those interested will therefore collect and send to the Herbarium at the Forest Research Institute, Dehra Dun, specimens of any plant they may find within the area, which is rare and little-known or has so far not been reported from the area, together with the field notes already referred to, these will not only be gratefully acknowledged and reported on but will also be mounted and carefully preserved for future reference.

It will not be out of place to mention here that while checking up herbarium specimens at the Dehra Dun Herbarium I have noticed that some plants have been wrongly assigned to our area by Duthie as a result of incorrect identification. For example, the inclusion of *Flacourtia sepiaria* Roxb. is based on a single specimen collected from Dehra Dun (Duthie No. 2264). This, however, is not a *Flacourtia* but almost certainly *Cudrania javanensis* Trecul. Again the inclusion of *Eurya symlocina* Bl. is based on a single sheet (Duthie 2618) collected from Birani-nadi, Dehra Dun. The specimen which unfortunately consists of barren leaves only looks exactly like *Myrsine semiserrata* Wall. Similarly the inclusion of *Strobilanthes pentastemonoides* T. Anders. is based on a wrongly identified specimen collected by U. N. Kanjilal from the banks of the Renaddi near Dehra Dun.

In conclusion it is my pleasant duty to thank Mr. C. E. Parkinson, Forest Botanist, for the kind help and guidance I have received from him in the preparation of the manuscript.

Ranunculaceae

Clematis Cadmia Ham.; Fl. Br. Ind. i, p. 2.

'Kusamhawa, Gorakhpur, Feb. 1913, P. C. Kanjilal Dehra Dun Herb. No. 52609! A climber.'

'Sakhui forests, Gorakhpur, 20th March 1920, Sri Ram, 2692! A climbing shrub.'

A slender climber with ternately decompose leaves and solitary axillary and bluish-white flowers; peduncles long with two leafy bracts about the middle. Achenes many, large, glabrescent, without feathery styles, with a short straight beak. It has been collected in grass lands, near water from Gorakhpur but seems to be rare. Flowers. Feb.-March. Fr. March-April.

Naravelia zeylanica DC.; Fl. Br. Ind. i, p. 7.

'Dogari, Haldwani division, 800 feet, 11. 1. 1927, A. E. Osmaston 1318! A climber.'

A more or less pubescent climbing shrub. Leaves 3-foliolate, the terminal leaflet transformed into a tendril. Flowers yellowish green, panicles lax, usually longer than leaves. Sepals 4, pubescent outside, soon deciduous. Petals 6-12. Achenes with long hairy styles.

Ranunculus laetus Wall.; Fl. Br. Ind. i, p. 19.

'Kaulagarh Tea Estate, Dehra Dun, May 1930, M. B. Raizada Dehra Dun Herb. No. 53543!'

An erect appressedly hairy perennial herb 1-2 feet high. Leaves 3-partite. Flowers 1 in. diameter, yellow. Achenes many in a globose head, not dotted, margined, rather large; style short, straight, broad at the base.

A single specimen of this Temperate Himalayan species was only once seen growing near a water channel in 1930 by the writer. It agrees fairly well with the description given in F. B. I. and the specimens so named in herbarium Dehra Dun.

Ranunculus muricatus Linn.; Fl. Br. Ind. i, p. 20.

'Chandbagh, Dehra Dun, 10-3-1926, B. L. Gupta Dehra Dun Herb. No. 41745! A weed.'

An erect, glabrous, annual herb, leaves 3-fid. Flowers 1/3-1/2 in. diameter, yellow. Achenes in a large, globose head, tubercled.

Ranunculus arvensis Linn.; Fl. Br. Ind. i, p. 20.

'Kaulagarh, Dehra Dun, January 1919, B. L. Gupta Dehra Dun Herb. No. 20384! Common in wheat fields.'

'New Forest, Dehra Dun, March 1930, M. B. Raizada Dehra Dun Herb. No. 51963!'

An erect nearly glabrous, much branched annual herb. Radical leaves cuneate 3-5-toothed, cauline 3-partite. Flowers 1/2 in. diameter, yellow. Achenes 5-10 densely spinous. An extremely common weed in cultivated fields during the cold weather.

Anonaceae

Uvaria Hamiltoni Hk. f. and Th.; Fl. Br. Ind. i, p. 48.

'Domakhand, Gorakhpur, 26-10-1914, Div. For. Officer, Dehra Dun Herb. No. 10115! A scandent shrub.'

'Domakhand, Gorakhpur, 9-4-1916, Sri Ram, 2479! A climber.'

An evergreen scandent shrub; young parts rusty tomentose. Leaves 4—10 in. long, 2—5 in. broad, pubescent above, stellately tomentose beneath. Flowers brick-red, about 2 in. across; pedicel 1 in. long, elongating in fruit. Ripe carpels baccate, ovoid, upto 1 in. across, rufous tomentose, scarlet, many seeded, borne on stalks 0.75—1.3 in. long. Flowers April-May. Fr. Rainy season.

Menispermaceae

Tinospora malabarica Miers.; Fl. Br. Ind. i, p. 96.

Fairly common throughout the area, usually climbing tall trees in cool situations.

This species has long been taken for *Tinospora cordifolia* Miers, which however differs principally in having smaller glabrous leaves, concave inner sepals and a smooth endocarp. Fl. February-March. Fr. May-June.

Capparidaceae

Crataeva lophosperma Kurz in Journ. Bot. iii (1874), p. 195.

'Gorakhpur, 11-4-1911, A. E. Osmaston Dehra Dun Herb. No. 2817!'

'Tilkonja, Gorakhpur, March 1914, P. C. Kanjilal Dehra Dun Herb. No. 52566! A tree.'

This species closely resembles *C. religiosa* Forst. f. but differs from it mainly in having usually 9-15 pairs of secondary nerves, berry 2-celled, seeds spinulose—tuberculate on the back; secondary nerves being 6-8 pairs, berry 1-celled, seeds smooth as in *C. religiosa*.

Caryophyllaceae

Sagina apetala Linn. Mant. ii, p. 559.

A slender, almost filiform wiry annual herb about 3 in. high. Stem ascending not rooting. Leaves opposite, subulate, connate at the base, filiform 0.2-0.5 in. long, spreading. Flowers small on capillary pedicels, green. Sepals 4 free. Petals none (or very minute), stamens 4, opposite the sepals and alternating with 4 very minute staminodes, ovary 1-celled, styles 4-5 minute, ovules many. Capsule 4-5-valved to the base.

Dehra Dun (Parker! Raizada!). A garden weed of damp places. Obviously imported from Europe. Fl. and Fr. cold season.

Portulacaceae

Talinum paniculatum Gaertn. Fruc. ii (1791), p. 219, t. 128.

Syn. *T. patens* Willd. sp. Pl. ii (1880), p. 863.

A glabrous, erect, succulent, somewhat shrubby herb. Stem almost simple 1-2 ft. high, leafy to the middle from where the panicle

begins. Leaves mostly opposite, ex-stipulate, oval, abruptly tapering towards the petioliform base 1·5-2 in. long, 0·7-1 in. broad. Panicle terminal, long, leafless bearing dichotomous cymes. Flowers carmine. Sepals two distinct about 0·2 in. long. Petals 5 rosy, ephemeral 0·25 in. long. Stamens about 15-20. Ovary free. Fruit a 3-valved capsule. Seeds black, minutely striolate.

Dehra Dun, self sown (Parker! Raizada!). Indigenous to the West Indies and east coast of S. America to Buenos Ayres. Fl. April-May. Fr. June-July.

Tamaricaceae

Tamarix Troupii Hole in Ind. For. XLV (1919), p. 248; Syn.

T. gallica Dyer in Fl. Br. Ind. i, p. 248 non Linn.

According to Hole the true *T. gallica* Linn. does not occur in India and that what has been hitherto called *T. gallica* in the plains of N. W. India is *T. Troupii* Hole.

Hypericaceae

Hypericum cernuum Roxb.; Fl. Br. Ind. i, p. 253.

Dehra Dun, on rocky cliffs (Raizada!).

An erect glabrous shrub; branches terete. Flowers 2 in. diameter, bright golden-yellow, 3-5 in. short terminal cymes. Petals obovate, longer than the stamens; styles twice the length of the ovary; capsule conical, 0·3-0·5 in. long. Fl. April-May.

Ternstroemiaceae

Actinidia callosa Lindl.; Fl. Br. Ind. i, p. 286.

Bindal Nala, Dehra Dun. Rather Scarce.

A sub-deciduous climbing shrub. Flowers white in pedunculate, axillary 1-7-flowered cymes, 0·5 in. diameter. Fruit a fleshy, ovoid berry, 0·8 in. long, edible. Fl. June. Fr. September.

Malvaceae

Abutilon avicennae Gaertn.; Fl. Br. Ind. i, p. 327.

'Domakhand, Gorakhpur, 28-4-1916, Sri Ram Dehra Dun Herb. No. 55852! A tall herb.'

A softly tomentose tall annual herb. Leaves orbicular-cordate; petiole 3 in. long. Peduncles 1 in., solitary, axillary. Flowers yellow. Carpels 15-20 much exceeding the sepals, oblong, truncate, with 2 long horizontally spreading awns. Fl. April.

Pavonia zeylanica Cav.; Fl. Br. Ind. i, p. 331.

'Akhagitgarh to Taragarh, Merwara, 14-1-86. Duthie 4535!'

'Etawah, U. P., 25-8-1921, R. S. Hole Dehra Dun Herb. No. 24998!'

'Jhansi, 12-11-1921, Sri Ram Dehra Dun Herb. No. 55853! and 55854!'

A glandular pubescent undershrub. Leaves small more or less 3-5-lobed. Peduncles 1-flowered upto 1.3 in. long. Flowers pink, about 0.4 in. long; bracteoles 8-9, setose, twice the length of the calyx. Ripe carpels glabrous, narrowly winged, enclosed in the persistent involucre of bracteoles. Fl. end of rainy season.

Tiliaceae

Grewia Hainesiana Hole in Ind. For XLIII (1917), p. 116; Syn. *G. asiatica* Roxb. non Linn.

Common throughout the Sub-Himalayan and Siwalik tracts of Northern India.

This plant according to Hole is not the same as *G. asiatica* Linn., which so far as at present known occurs only in cultivation, it is doubtfully wild in India and even in cultivation, it is not at all common within our area. This tree (*G. Hainesiana*) has more or less correctly been described by Duthie on page 113 of his Flora but under the name of *G. asiatica* Linn.

Dr. M. Burret describes in Notizblatt Botanischen Gartens und Museums Berlin-Dahlem IX, p. 663, a new species of *Grewia*, *G. mesopoda* Burret from Lachiwala (Dehra Dun). I have not seen his type specimen but judging from the locality I have no doubt that his species could not be anything but *G. Hainesiana* Hole.

Geraniaceae

Oxalis latifolia H. B. and K. Nov. Gen. et Sp. V (1821), p. 184, t. 467.

A herb about 6-10 in. high, stemless, with 2-6 long-petioled radical ternate leaves and 2-3 scapes all arising from a single bulb. Petioles slender, 5-9 in. long, puberulous. Leaves trifoliate, with almost sessile leaflets; leaflets equal, broadly deltoid, sub-bilobed with lobes divergent and acute, broadly but distinctly cuneate at the base, 0.6 in. long, 1.2 in. broad. Scapes generally 1 or 2, filiform, 5-10 in. long; umbels 5-6 flowered, bracteate. Flowers pedicelled 0.4 in. long; pedicels slender. Sepals 5, equal, elliptic-oblong 0.2 in. long, glabrous, distinctly 5-nerved, persistent, bi-glandular towards the apex; glands narrow, contiguous, not divergent. Petals 5, violet, united upto a fourth of their length. Stamens 10, alternately longer and shorter, united below into a membranous cup. Ovary elongate, almost 5-lobed; styles 5 distinct. Ovules 4 in each cell.

A native of Mexico but now completely naturalized in shady places in Dehra Dun. Fl. May-July.

Oxalis corymbosa DC. Pd. i (1824), p. 696.

A perennial herb about 10 in. or more high, with the petioles arising directly from the bulb. Leaves 9-12, basilar, stipulate, trifoliate; petioles very thin, flexuous, 5-10 in. long; leaflets equal, broadly obcordate with a narrow sinus, base cuneate, 0.5-1 in. long, 0.7-1.4 in. broad, membranaceous, adpressed hairy on both surfaces, punctate with black glands. Scapes 2-3 very long and flexuous, 6-10 in. long, longer than the petioles. Cymes umbelliform, cernuous, 6-7-flowered, bracteate. Flowers about 0.6 in. long, pedicellate, pedicels unequal, slender, 0.2-0.7 in. long. Sepals 5, distinct, with two small orange-coloured divergent glands at the apex. Petals 5, violet. Stamens 10, filaments united for about a third of the distance from the base into a short angular tube, alternately longer and shorter. Ovary obtusely 5-angled; styles 5 distinct.

Dehra Dun, naturalized. A native of Tropical America. Fl. May-July.

Hydrocera triflora W. & A.; Fl. Br. Ind. i, p. 483.

'Bargad chauki, Pilibhit, 24-6-1902, Inayat 25862!'

An erect, glabrous, annual water-weed with fistular floating stems rooting at the nodes, linear lanceolate leaves and red globose succulent fruit.

Meliaceae

Dysoxylum binectariferum Hk. f.; Br. Ind. i, p. 546.

'Haldwani division, U. P., September 1922, H. G. Champion Dehra Dun Herb. No. 32888! 32889!'

Pilapani, Haldwani div., 650 feet, 2-12-1925, A. E. Osmaston 1227! A small tree.'

A tree about 50 feet high; young shoots and inflorescence minutely puberulous. Leaves alternate 12-24 in. long, imparipinnate. Leaflets 6-11, alternate. Flowers 0.3-0.4 in. diameter in terminal panicles 2-4 in. long. Capsule 1.5-2 in. diameter, orange, globose or pyriform containing 1-3 large, black seeds enclosed in a scarlet aril.

Olacaceae

Natsiatum herpeticum Ham.; Fl. Br. Ind. i, p. 595.

'Dogari, Haldwani div., U. P., 800 feet, 11th January 1927 A. E. Osmaston 1317! A slender climber, stem somewhat woody at base.'

'Senapani, Haldwani, January 1928, H. G. Champion Dehra Dun Herb. No. 45243!'

A slender climbing shrub; leaves alternate, petiolate, cordate-ovate, 7-9-nerved. Racemes supra-axillary, long, pendulous. Flowers dioecious, greenish-yellow, minute, with a foetid smell. Fruit a small, slightly fleshy drupe, black when ripe. Fl. January; Fr. April.

Celastraceae

Gymnosporia Falconeri Lawson; Fl. Br. Ind. i, p. 620.

Fairly common throughout the Sub-Himalayan tract in Ramnagar and Haldwani division. Some specimens collected by Mr. H. G. Champion from the Ramnagar division have recently been described in the Kew Bull. 1921, p. 308, as *G. Championi* Dunn, but I am unable to distinguish them from other specimens of *G. Falconeri*.

An erect evergreen shrub 4-12 feet high. Leaves 1.25-3.5 in. long, 0.5-2 in. broad, elliptic or ovate, serrulate, clothed on both surfaces with short white pubescence which is denser beneath. Flowers small, in fascicled axillary few-flowered cymes. Capsule 0.3-0.4 in. long, turbinate, 3-celled, slightly lobed, smooth, brown. Seeds enclosed in a white aril. Fl. March. Fr. December.

Salacia prinoides DC.; Fl. Br. Ind. i, p. 626.

Banki forests, Gorakhpur, 27th January 1918, Sri Ram Dehra Dun Herb. No. 52594! A large woody climber'.

A large evergreen woody climber. Leaves opposite, 2-3 in. long, coriaceous, oblong or elliptic, serrulate. Flowers 2-6 clustered together on axillary tubercles, pale yellow about .25 in. across. Fruit baccate, scarlet when ripe, globose up to 0.5 in. across, usually 1-seeded. Fl. December-January; Fr. Cold season. The erect form of this plant has not been recorded from our area.

Rhamnaceae

Zizyphus hysudrica Hole in Ind. For. XLIV (1918), p. 505.
Syn. *Z. jujuba* Lamk. var. *hysudrica* Edgew. in Journ. Linn. Soc. VI (1862), p. 201-202.

A medium sized evergreen tree often attaining a large girth; branches mostly erect, spines very variable, large and in sub-equal pairs on young plants, often wanting on older trees. Leaves 1.5-2.5 in. long or smaller on young plants, from elliptic to broadly ovate or orbicular, rounded at both ends often oblique at the base, entire or serrulate, glabrous above, glabrescent beneath, strongly 3-nerved, nerves prominent above; petiole 0.2-0.7 in. long. Flowers as in *Z. jujuba* Lamk. Drupe 0.75 in. diameter in the wild form, globose, greenish-red when ripe.

Ajmere, 16-10-1887, Duthie 6623! 6624!

Vitaceae

Vitis auriculata Roxb.; Fl. Br. Ind. i, p. 658.

'Kalyanpur, Banda, 24-10-1916, Sri Ram Dehra Dun Herb. No. 52547! A climbing shrub'.

'Kalyanpur, Manikpur Range, South Banda, 1-10-1921, P. C. Kanjilal Dehra Dun Herb. No. 52546! 52548!'

A soft-wooded climber with corky bark when old. Tendrils 2-fid. Leaves digitately 3-5-foliolate. Leaflets 2-6 in. long, terminal largest, glabrescent above, pubescent beneath. Inflorescence axillary on long peduncled divaricating compound cymes. Flowers small, bisexual, tetramerous, greenish. Berry 0.7 in. across, roundish, 1-seeded. Fl. rainy season. Fr. October.

The record of this species is based on somewhat imperfect material in Herb. Dehra Dun.

Vitis Parkeri Gagnepain ex Osmaston in For. Fl. Kumaon (1927), p. 120.

'Garjia, Ramnagar div., U. P., 1,500 feet, 13th January 1922, A. E. Osmaston 1175! A fairly large climber.'

'Guliapani Block, Haldwani div., 900 feet, 6-12-1925, A. E. Osmaston 1284! A large climber'.

A fairly large, soft wooded climber with terete stem and corky bark. All parts, except the inflorescence glabrous. Tendrils leaf-opposed, usually simple, often stout and long. Leaves digitately 3-6 foliolate, never pedate. Petiole 3-8 in. long, terete. Leaflets 2-7 in. long, 1.2-4 in. broad, terminal longest. Flowers minute, dioecious, tetramerous, in axillary, divaricate, subcorymbose compound cymes, up to 3 in. long, common peduncle branched near the base. Fruit a berry 0.4-0.8 in. long, oblong or ovoid, reddish at first but finally black. Seeds usually 1 or up to 3 ellipsoid, bluntly beaked, marked with parallel horizontal furrows laterally and with a groove on the face and another almost continuous one on the back, the latter with a low ridge. Fl. November-January. Fr. April-May.

Bibliography

A detailed bibliography would be too lengthy for inclusion here, but the following works, which I have freely consulted, among others, in the preparation of this paper, may be mentioned:

BRANDIS.—Indian Trees.

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HUTCHINSON.—Families of Flowering Plants.

KANJILAL.—Forest Flora for Pilibhit, Oudh, Gorakhpur and Bundelkhand.

OSMASTON.—Forest Flora for Kumaon.

PARKER.—Forest Flora for the Punjab, with Hazara and Delhi.

NUMBER OF CHROMOSOMES IN *SUAEDA FRUTICOSA* FORSK.

BY

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Received for publication on 10th September, 1934.

Chromosome numbers are already known in several Chenopodiaceæ due to the work of several investigators.* In the present note the number of chromosomes in *Suaeda fruticosa* Forsk., another member of the same family, is being reported. These have been counted from the apical meristem of the shoot in seedlings of the species which had been raised from seeds collected at Lahore some four years ago.

The cells occupying the apical growing point of the stem were found to yield all stages of mitosis and 36 chromosomes were counted in several instances during late prophase, just before metaphase. They are seen at this time to be small somewhat elongated bodies with longitudinal splits. During metaphase the chromosomes are mostly clumped up and it is not possible to count them clearly, but during telophase their number can again be determined when the daughter nuclei are organized. Even during interphase in cells undergoing rapid division the chromosomes remain distinct and it is possible to count their number.

* For this, reference may be made to the papers of Tischler (Tab. Biol. VII, 1931) and Billings (Bot. Gaz. XCV, 1934). The accompanying list of chromosome numbers in the Chenopodiaceæ has also been drawn up from these publications and the names of the original investigators are given therein.

The other Chenopodiaceæ in which the chromosome numbers are known are the following:—

Species.	N Chromosomes.
<i>Chenopodium album</i>	9
<i>C. hybridum</i>	9
<i>C. murale</i>	9
<i>C. vulvaria</i>	9
<i>C. Bonus-Henricus</i>	18
<i>Atriplex hastatum</i>	9
<i>A. litorale</i>	9
<i>A. hortensis</i>	9
<i>A. patulum</i>	18
<i>A. hymenelytra</i>	9 and 10*
<i>Beta vulgaris</i>	9
<i>B. maritima</i>	9
<i>B. trigyna</i>	27
<i>Bassia hirsuta</i>	9
<i>Spinacea oleracea</i>	6
<i>Hablitzia tamnoides</i>	9

In *Suaeda fruticosa*, as the diploid number is 36, the haploid may be calculated to be 18, and is thus the same as in *Chenopodium Bonus-Henricus* and *Atriplex patulum*.

In the family Amarantaceæ, chromosome numbers are not known in many forms. The author has been able to find only two species in which these have been reported so far. In *Celosia cristata*, the haploid number of chromosomes is 18 according to the investigations of Morinaga, Fukushima, Kano, Maruyama and Yamasaki (Bot. Magaz., Tokyo, 43: 589, 1929); and in *Digera arvensis*, the N chromosomes are only 6, the same as in *Spinacea oleracea* in Chenopodiaceæ (Joshi and Rao: Jour. Ind. Bot. Soc., XIII, 1934). Even this, however, is enough to show that the chromosome numbers in the families Chenopodiaceæ and Amarantaceæ show very close correspondence, and cytological evidence fully supports the close affinity of the two groups, which has already been recognised by everybody on grounds of both floral morphology and vegetative anatomy.

* *Atriplex hymenelytra* is a dioecious species, and according to Billings (l. c.), the male plants during the heterotypic division show eight bivalents and a tripartite XY group on the metaphase plate. The X element is composed of two separate chromosomes. The N count is, therefore, both 9 and 10.

**A REPLY TO THE POST-SCRIPT OF THE PAPER
ON "STUDIES IN THE FAMILY ALISMACEAE.**

1. LIMNOPHYTON OBTUSIFOLIUM, MIQ." by

Brij Mohan Johri

BY

S. K. NARASIMHA MURTHY

Department of Botany, Central College, Bangalore

Received for publication on 3rd September, 1935.

There is a difference of opinion between Mr. Johri and myself regarding the number of nuclei in the embryo-sac of *Linnophyton obtusifolium*. He holds that it is primarily six-nucleate and regards the eight-nucleate condition as being exceptional. According to my observations the embryo-sac is primarily eight-nucleate during which stage it is much shorter and more or less straight. This is rapidly followed by an elongation of the embryo-sac accompanied by its bending at the chalazal end and the disorganisation of two of the nuclei thus resulting in a six-nucleate condition. As the eight-nucleate stage is passed through very rapidly, one is often misled to interpret that the embryo-sac has only six nuclei while actually it is a derived condition.

We differ again as regards the development of the fertilised egg. He observes that the division of the fertilised egg is always preceded by the division of the primary endosperm nucleus. But I usually find that the fertilised egg is precocious in its development and divides prior to the division of the primary endosperm nucleus.

I entirely agree with him in the fact that the nucellus is not disorganised early.

A NOTE ON THE ANDROECIUM OF *BOMBAX MALABARICUM* DC.

BY

J. VENKATESWARLU

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Received for publication on 26th August, 1935.

The occurrence of stamens with thicker filaments and bilocular anthers in *Bombax malabaricum* DC., besides others with unilocular anthers, has been recently recorded by Edlin*, but no mention has been made by this writer or in earlier literature about their number and exact position in a flower. The present note gives these details.

The occurrence of a few stamens with bilocular anthers seems to be a character common to all the flowers of *Bombax malabaricum*. It is at least so in the trees growing in the Benares Hindu University grounds. It is seen that in all the flowers from each of the five groups of stamens into which the androecium is divided in this species, some of the inner stamens are turned towards the centre and closely clasp the style. Such stamens are mostly 15 in number and out of these about five have thicker filaments which bear bilocular anthers.

* Edlin, H. L., A critical revision of the Taxonomic groups of Malvales. New Phytologist, Vol. XXXIV, No. 1, 1935.

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